Standard level determination of pungency in Sambal Terasi (case of study: Warung Makan Betawi micro small medium enterprises)

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Abstract. Sambal Terasi is a hot sauce that uses good chili as main ingredients and added terasi (fermented shrimp or fish) as an additional spice. The primary taste of Sambal Terasi is spicy. It occurs because of capsaicin contained in chili. Today, the problem of many Micro, Small and Medium Enterprises (MSME) in Indonesia is they have not quantified the standard level of pungency in Sambal Terasi based on the scientific method, but it relied on human subjectivity. This research aims to quantify the Scoville Scale of the pungency of Sambal Terasi at various levels in Warung Makan Betawi MSME. The research methods were descriptive research method on the composition of Sambal Terasi and quantitation of capsaicin concentration (g/g) using High-Performance Liquid Chromatography, then converted into Scoville Heat Units (SHU). The results have shown that standardized composition, ingredients and procedures are important to equalize the pungency of each production of Sambal Terasi. Scoville Scale and classifications for each level of Sambal Terasi were as follows: level 1 has 1065 SHU as non-pungent; level 2 has 2262 SHU as mildly pungent; level 3 has 3619 SHU as moderately pungent; level 4 has 3957 SHU as pungent; level 5 has 5215 SHU as highly pungent.

Keywords: Sambal Terasi, capsaicin, Scoville heat units, high performance liquid chromatography, pungency

1. Introduction
Chili pepper is a plant of the Solanaceae family which belongs to the genus Capsicum. It originated from the American continent of Peru and spread throughout the world. These plants are very famous in the world because of their special characteristics of smell and taste [1].

One kind of product that can be produced from chili pepper is sambal. Sambal is a sauce obtained from good chili pepper ingredients, then processed using additional spices with or without the addition of other foods [2]. Every additional spice that added into sambal must be the food that is allowed by the government.

There are so many types of sambal that are available today, and Indonesia is a country that has a lot of types of sambal. Each region in Indonesia has its own special sambal, for example, Sambal Matah from Bali, Sambal Ijo from Padang, Sambal Bawang, starfruit black chili sauce, and Sambal Terasi Ijo.
Each type of sambal also has different recipes depending on the person who produces it, so that each sub-variant of sambal has its own distinctive taste characteristics.

From various types of sambal, Sambal Terasi has been selected as the main focus of this research. Sambal Terasi is a hot sauce obtained from good chili ingredients that added terasi as an additional spice. *Terasi* or *Belacan* is fermented products from Indonesia derived from fish and fresh shrimp [3].

Sambal Terasi selected because it has been widely known in Indonesia and produced by large industries so that it will easy to introduce this product to the public. The examples of a large industry which produces Sambal Terasi are PT HEINZ ABC with product name Sambal Terasi ABC and FINNA FOOD with product name Sambal Terasi Uleg.

Besides large industries, there are many micro, small, and medium enterprises (MSMEs) produce Sambal Terasi. One of these enterprises is Warung Makan Betawi. This enterprise is becoming a case of study of this research. The reasons for choosing Sambal Terasi recipe from Warung Makan Betawi are the taste that has survived for years; consistency in the recipe and Sambal Terasi has been produced continuously. Determining the standard of pungency in sambal is something that already done. Some sambal production sites do have their own standards for spiciness. The standard is expressed in the form of levels. The higher the level, then sambal will be spicier. However, the measurement of the pungency of sambal at various levels is not based on the scientific method. Measurements are more qualitative, and the results tend to be subjective.

The spicy flavor from sambal itself caused by the main ingredient, which is chili pepper containing capsaicin as an active compound. Capsaicin is a group of alkaloids [4]. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) has the characteristics of lipophilic, colorless, odorless with the molecular formula $C_{18}H_{27}NO_3$ and molecular weight of 305.40 g/mol [4].

The first scientific method for measurement of chili pepper pungency was Scoville Organoleptic Test. This method was created by American scientist Wilbur Scoville [5]. In this method, capsaicin is extracted with alcohol solvents. After that, the chili solution is mixed with a sugar-water solution until the spicy taste of the solution is no longer detected by the tongue [6].

The level of dilution of the extract with sugar water solution gives a Scoville unit, which represents the level of spiciness of chili pepper from various variants, the higher the Scoville number, the higher the spiciness rate of chili itself [5]. This method is a standard for measuring the level of the pungency of chili, but this method is considered less effective because the measurement is subjective. The difference in panelists who measure will have different measurements of spiciness. Therefore, that traditional method has been replaced by instrumental methods [7].

One type of instrumental methods is HPLC (High Performance Liquid Chromatography). This method is claimed to be more accurate and reliable than traditional methods that use the human senses. The result of this method is capsaicin concentration, which can be converted into Scoville Heat Units, so that later when measurements have been made, it can be standardized well and understood by all people [8]. This research aims to determine heat pungency in Sambal Terasi at various levels which produced by Warung Makan Betawi.

2. Materials and methods

2.1. Materials

Sambal Terasi recipe was collected from Warung Makan Betawi. The ingredients such as chili, tomatoes, terasi, sugar, salt, flavoring, and cooking oil were purchased from Pasar Timbul at Moch Kahfi 1 Street, South Jakarta, Indonesia. Reagent such as methanol was provided by Central Laboratory Padjadjaran University.

2.2. Sambal production

First, raw materials such as curly red chili, cayenne pepper, tomatoes, terasi, sugar, salt, flavoring, and cooking oil prepared. After raw materials preparation, the ingredients were sorted according to the standard criteria of ingredients. Then the material is washed and weighed. Cooking oil heated based on
standard temperature, then put terasi, curly red chilies, cayenne peppers, and tomatoes into a frying pan. After that, sugar, salt and flavoring added. If Sambal Terasi was cooked according to the indicator, then it was smoothed using a blender. After smooth, Sambal Terasi poured into a glass jar and stored in a refrigerator.

2.3. Extraction of capsaicin
A sample of 1 gram was weighed. Put in a 50 ml beaker glass, then add 20 ml of Methanol. The sample was then sonicated for 30 minutes using a sonicator. Then, the sample was filtered using filter paper. A 1 mL aliquot of each Sambal Terasi was transferred into a 25 mL volumetric flask. Methanol was added to the limit as a dilution of the concentration. Samples were ready to be tested using HPLC to measure capsaicin concentration.

2.4. High performance liquid chromatography analysis
High Performance Liquid Chromatography was provided by Central Laboratory Padjadjaran University. The HPLC conditions were as follows:

1. HPLC brand Alliance® HPLC - e2695 Separations Module.
2. Sample volume: 10 μL, flow rate: 1 ml/minute.
3. Max Pressure: 1374 psi, Min: 1359 psi.
4. UV detection wavelength at 227 nm.
5. Mobile phase: 30% water, acetonitrile 40%, methanol 30%.

![Figure 1. Calibration curve for capsaicin.](image)

Standard solutions were prepared from a stock solution of capsaicin using a variation of dilutions at 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The standard solutions were run on the high-performance liquid chromatography. The obtained standard curve plots of peak area against concentration are shown in figure 1. During HPLC sample analyses, a standard solution must be injected every 10 samples to evaluating the retention time reproducibility and instrument calibration [9]. Every sample of sambal was injected twice.

2.5. Conversion to Scoville Heat Units (SHU)
Based on the generally accepted Scoville Organoleptic Test, the spiciness of the chili sample was calculated by converting capsaicin concentration represented by grams of capsaicin/gram of chili [9-10]. This formula was modified into a gram of capsaicin/gram of sambal. The formula for this conversion was as follow:

\[
\text{Scoville Heat Unit} = \text{Capsaicin Concentration (g/g)} \times (1.6 \times 10^7) \tag{1}
\]
The results of the conversion are matched with the classification according to international standards. This classification was as follows [11]: (0-700 SHU) non-pungent, (700-3000 SHU) mildly pungent, (3000-25000 SHU) moderately pungent, (25000-70000 SHU) highly pungent, (> 80000 SHU) very highly pungent.

2.6. Statistical analysis
The data that has been obtained consists of the Scoville scale were analyzed using t-test: paired two samples for means, which is a technique in statistics that uses for the significance of the difference in the two averages needed from two different distributions. This t-test analysis was used Microsoft Excel program. If –t Stat < t Critical two-tail or t Critical two-tail < t Stat, then the difference of pungency between the levels of Sambal Terasi is significant.

3. Results and discussion

3.1. How to determine heat pungency in sambal at various level
Each type of sauce has its own uniqueness both in terms of color, taste, and level of spiciness. Sambal with the same type does not necessarily have the same taste. Each micro, small, medium enterprises have their own unique recipe for sambal production. This difference in production and recipe will affect the heat of spiciness in sambal.

In order to be able to approach the similarity of taste and spiciness, then it is necessary to standardize composition, ingredients, and procedures. This standard helps Warung Makan Betawi to produce Sambal Terasi as they expected.

This paper also explained standard recipes, ingredients, and procedures to make Sambal Terasi (Chili sauce with fermented shrimp). Therefore, all of the standard which has been written in this paper can be copied to produce Sambal Terasi with the same taste and pungency as Warung Makan Betawi have had.

3.2. Standard of composition
The level of the chili paste is made up of 5 levels. Sambal Terasi made from level 1 to level 5 with the extra addition of cayenne pepper every increase in level. Table 1 describes the standard composition of Sambal Terasi.

| Ingredients          | LV1 (grams) | %   | LV2 (grams) | %   | LV3 (grams) | %   | LV4 (grams) | %   | LV5 (grams) | %   |
|----------------------|-------------|-----|-------------|-----|-------------|-----|-------------|-----|-------------|-----|
| Curly red chili      | 200         | 35.9| 200         | 32.9| 200         | 30.4| 200         | 28.3| 200         | 26.4|
| Cayenne pepper       | 0           | 0   | 50          | 8.2 | 100         | 15.2| 150         | 21.2| 200         | 26.4|
| Terasi Medan         | 22          | 3.9 | 22          | 3.6 | 22          | 3.3 | 22          | 3.1 | 22          | 2.9 |
| Salt                 | 7           | 1.3 | 7           | 1.2 | 7           | 1.1 | 7           | 1.0 | 7           | 0.9 |
| Flavouring (Sasa)    | 1.5         | 0.3 | 1.5         | 0.2 | 1.5         | 0.2 | 1.5         | 0.2 | 1.5         | 0.2 |
| Sugar                | 17          | 3.0 | 17          | 2.8 | 17          | 2.6 | 17          | 2.4 | 17          | 2.2 |
| AGATHA F1 Tomatoes   | 150         | 26.9| 150         | 24.7| 150         | 22.8| 150         | 21.2| 150         | 19.8|
| Cooking oil          | 160         | 28.7| 160         | 26.3| 160         | 24.3| 160         | 22.6| 160         | 21.1|
| Total                | 557.5       | 100 | 607.5       | 100 | 657.5       | 100 | 707.5       | 100 | 757.5       | 100 |

3.3. Ingredients criteria
Raw materials standard is necessary to produce the same taste of each production. This standard can include varieties of material, level of maturity, content in ingredients, size of material and other criteria that are deemed necessary. In this case, the raw material is standardized as a chili paste from Warung Makan Betawi. Standardized ingredients include curly red chili, cayenne pepper, tomatoes, terasi, salt, flavoring, sugar and cooking oil.
3.3.1. *Curly red chili.* Curly red chili that is used is the Lembang-1 variety. The fruit length and diameter minimum are 5 cm for length and 0.5 cm for diameter. The color of chili also must be standardized. The color range for the standard is HSL (Hue, Saturation, Lightness). Hue represents the color selection of the color wheel. Saturation represents a measure of the purity of a color. Lightness represents the light scale from the darkest (black) to the brightest (white). The color standards for red chili are as follows:

Table 2. Color standard.

| HSL          | Range          |
|--------------|----------------|
| Hue          | 1° - 2°        |
| Saturation   | 77% - 89%      |
| Lightness    | 34% - 46%      |

The color of the chili that is generally seen is red. Then the color space in table 2 illustrates the types of red seen in curly red chili.

3.3.2. *Cayenne pepper.* The cayenne pepper used is the rabani agrihorti variety. This type of chili originates from Lembang, Bandung. The minimum standard length for this chili is 3 cm, with a minimum diameter is 0.5 mm. Standard colors to determine the level of maturity using the HSL color range. The HSL composition is shown in table 3.

Table 3. Cayenne pepper color standard.

| HSL          | Range          |
|--------------|----------------|
| Hue          | 3° - 22°       |
| Saturation   | 100%           |
| Lightness    | 50% - 60%      |

3.3.3. *Tomato.* The selected tomatoes are F1 Aghata varieties. The weight of this tomato ranges from 145 grams to 150 grams. Unlike chili, this type of tomato has a nearly uniform size. So, it does not require minimum length and minimum diameter. The maturity level of tomatoes can also be measured from the color of the fruit. The maturity level of tomatoes based on color range is shown in table 4.

Table 4. Tomato standard color.

| HSL          | Range          |
|--------------|----------------|
| Hue          | 10° - 32°      |
| Saturation   | 75% - 76%      |
| Lightness    | 42% - 50%      |

3.3.4. *Terasi standard.* Terasi that used in Sambal is Terasi Medan (Fermented shrimp made from Medan City, Indonesia). Terasi Medan is a *terasi* from Rebon shrimp. This King of terasi Ade their Oen brand. The name of this Band si Juex. The ingredients in *terasi* is shown in table 5.

Table 5. *Terasi* standard content based on label.

| Content       | Percentage  |
|---------------|-------------|
| Protein       | Min 20%     |
| Fat           | Min 2.5%    |
| NaCl          | Min 16%     |
| Moisture      | Min 30%     |
| Acid Insoluble Ash | Max 1.5% |
3.3.5. **Salt standard.** The variety of salt that is used is stone salt. Stone salt is different from powdered salt. This type of salt has the composition of NaCl and KIO₃. The brand of salt that is used for produce Sambal is the WM brand. Based on the label, KIO₃ substances contained in this salt are at least 30 ppm.

3.3.6. **Flavoring (Sasa brand).** The flavoring used serves as a trigger for savory flavors in sambal. This flavoring is made from sugar cane extract, which is processed by fermentation. The brand of flavoring that is used for produce sambal is the WM brand. Based on the label, this flavoring contains 99% monosodium glutamate.

3.3.7. **Sugar standard.** The sugar used is sugar made from sugarcane water. There are no specific criteria for sugar.

3.3.8. **Cooking oil.** Cooking oil that is used for Sambal Terasi is palm cooking oil. The brand of cooking oil is Filma. This cooking oil should not be used more than once.

3.4. **Standard procedure to produce Sambal Terasi**

Just like the standard criteria for raw materials, determining the standard in the procedure is very important to do. The ingredients like curly red chili, cayenne pepper, tomatoes, and terasi are first prepared in 1 container. The first thing to do is to heat the oil that has been weighed before. The heat for the initial cooking standard is 76 °C, but after being in the cooking process, the temperature must be kept between 76 °C-86 °C. The temperature is quite influential on the concentration of capsaicin in the product [12]. Therefore temperature is a factor that must be considered.

After the oil reaches the standard initial cooking temperature, then terasi, tomatoes, curly red chili, cayenne pepper, tomatoes are put together into the frying pan. After 2 minutes of cooking, then salt, flavoring and sugar are added. Stir the ingredients every 1.5 minutes. The function of the stirring process is to prevent material clumping and heat can be spread evenly during the cooking process. An indicator that shows that cooking must be stopped is a visual change in tomato skin. Tomatoes that lose water content during cooking will show changes to their skin, which is shrinking, and the skin on the tomatoes becomes wrinkled.

The next step is the cooked ingredients are cooled to room temperature for 5 minutes. After that, the ingredients are blended using a blender for 4 minutes. Sambal, which has been smooth, put into a jar. It should be noted that the difference in packaging affects the amount of existing capsaicin [12]. After putting into a jar, keep sambal until it reaches room temperature (25°C). Then, close the jar tightly. Each level of chili sauce is then put into the refrigerator together. The storage temperature is set at 2 °C-8 °C so that the capsaicin condition remains stable during the storage process before analyzing [13].

3.5. **Analysis of capsaicin concentration in samples**

Sample of sambal was then injected twice into the HPLC. After obtaining the area, then it is included in the regression equation to get the concentration in units of ppm. The results of this regression equation must be multiplied by the dilution factor and divided by the sample weight. Then the capsaicin concentration in the actual sample is obtained. After that, it is changed from ppm to g/g sample. Results can be seen in table 6.
Table 6. Capsaicin concentration in sambal.

| Level | Cayenne Pepper (grams) | Concentration (ppm) | Standard Deviation |
|-------|------------------------|---------------------|--------------------|
| 1     | 0                      | 66.5316             | 0.1911             |
| 2     | 50                     | 141.3895            | 0.3055             |
| 3     | 100                    | 226.2094            | 0.0781             |
| 4     | 150                    | 247.3406            | 0.1228             |
| 5     | 200                    | 325.9618            | 0.1744             |

Based on table 6 it can be seen that the average concentration of capsaicin in level 1 sambal is 66.5316 ppm; level 2 is 141.3895 ppm; level 3 is 226.2094 ppm; level 4 is 247.3406 ppm; level 5 is 325.9618 ppm. It can be concluded that there is an increase in the concentration of capsaicin at each level increase in the chili sauce. The difference between the level 1 concentration to level 2 is 74.8580 ppm, level 2 to level 3 is 84.8199 ppm, level 3 to level 4 is 21.1312 ppm and level 4 to level 5 is 78.6212 ppm. There is an anomaly from level 3 to level 4. The difference in concentration is even less than 50 ppm. But it has not yet been determined whether this small concentration difference means there is no difference in the level of spiciness. It needs to be converted first to the Scoville unit.

3.6. Sambal Terasi pungency based on Scoville Heat Unit

The concentration (ppm) must be converted into gram/gram. Then, the results of this unit were converted to scoville scale by multiplying the capsaicin level with the spiciness coefficient for pure capsaicin by $1.6 \times 10^7$ [10]. Table 7 shows the results of the level of spiciness of Sambal Terasi from level 1 to level 5.

Table 7. Scoville scale of each level in Sambal Terasi.

| Level | Concentration (ppm) | Concentration (g/g) | Scoville Heat Units (SHU) |
|-------|---------------------|---------------------|---------------------------|
| 1     | 66.5316             | 0.00006653          | 1065                      |
| 2     | 141.3895            | 0.00014139          | 2262                      |
| 3     | 226.2094            | 0.00022621          | 3619                      |
| 4     | 247.3406            | 0.00024734          | 3957                      |
| 5     | 325.9618            | 0.00032596          | 5215                      |

After obtaining Scoville Heat Units, we can classify each level into the existing international classification based on SHU. The classification is shown in table 8.

Table 8. Classification of Sambal Terasi based on existing international classification.

| Level | SHU | Classification     |
|-------|-----|--------------------|
| 1     | 1065| Mildly pungent     |
| 2     | 2262| Mildly pungent     |
| 3     | 3619| Moderately pungent |
| 4     | 3957| Moderately pungent |
| 5     | 5215| Moderately pungent |

Table 8 presented that Sambal Terasi at levels 1 and 2 are in the same classification, which is mildly pungent. Whereas Sambal Terasi at levels 3, 4 and 5 are classified as moderately pungent. The range of each grouping is indeed quite large, for example moderately pungent classification in the range of 3000-25000 SHU [11]. This caused the classification between levels 3, 4 and 5 are all the same.
To further analyze the differences in pungency at different levels significantly or not, a sample t-test analysis was conducted. The level of trust tested is 95%. T-test analysis using Microsoft Excel. The results of the analysis can be seen in table 9.

**Table 9.** Significance analysis of Sambal Terasi.

| Compared Level | First Scoville Scale (SHU) | Second Scoville Scale (SHU) | Difference between first and second Scoville Scale (SHU) | Result of t-test |
|----------------|-----------------------------|-----------------------------|----------------------------------------------------------|------------------|
| 1 and 2        | 1065                        | 2262                        | 1198                                                     | Significantly different |
| 2 and 3        | 2262                        | 3619                        | 1357                                                     | Significantly different |
| 3 and 4        | 3619                        | 3957                        | 338                                                      | Significantly different |
| 4 and 5        | 3957                        | 5215                        | 1258                                                     | Significantly different |

From table 9, it can be seen that each Scoville Scale increase in the level of Sambal Terasi has a statistically significant difference. Although there is a small Scoville Scale increase between level 3 and level 4, it can be said to be significantly different. Because the difference in each level is significantly different, a new standard for spiciness level classification for Warung Makan Betawi can be made based on known levels of SHU value. The classification starts from non-pungent, mildly pungent, moderately pungent, pungent and very pungent as shown in table 10.

**Table 10.** Classification level of Sambal Terasi based on results of statistical analysis for Rumah Makan Betawi.

| Level | Capsaicin Content (ppm) | Scoville Scale (SHU) | Classification |
|-------|--------------------------|----------------------|----------------|
| 1     | 66.5316                  | 1065                 | non-pungent    |
| 2     | 141.3895                 | 2262                 | mildly pungent |
| 3     | 226.2094                 | 3619                 | moderately pungent |
| 4     | 247.3406                 | 3957                 | Pungent        |
| 5     | 325.9618                 | 5215                 | very pungent   |

Every person has a different sensitivity to spiciness; therefore it will be more accurate to measure spiciness with an instrument, such as HPLC. Determined classification from this research will be a reference for someone in measuring the ability to receive a spicy taste.

4. Conclusions

Based on the results of the research and analysis in result and discussion, it can be concluded that to determine the pungency at various levels of Sambal Terasi is standardizing the composition, raw materials, procedures, and also quantitative analysis using HPLC.

Scoville Scale and classifications for each level of Sambal Terasi were as follows: level 1 has 1065 SHU as non-pungent; level 2 has 2262 SHU as mildly pungent; level 3 has 3619 SHU as moderately pungent; level 4 has 3957 SHU as pungent; level 5 has 5215 SHU as highly pungent.

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