Effects of The Addition of Complexing Agents on Curcumin Stability Using Accelerated Shelf Life Testing

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Abstract. Curcumin as a natural colorant product from turmeric used in food industry has low stability. Curcumin is easily oxidized due to light, oxygen, or high temperature. One of the methods that can be used to increase its stability is by complexation process using metal ions. The aim of this research are to determine product shelf life and the best complexing agent to increase curcumin stability. In this research there were three complexing agent used to form curcumin complex. Then, the stability of products are measured through curcumin concentration parameter and based on its shelf life using Accelerated Shelf Life Testing (ASLT) method. The results stated that curcumin-Zn complex exerted the highest stability against control and other complexing agent, hence the shelf life was projected to increase by 10-15 days at 35°C compared to untreated liquid curcumin. Conversely curcumin-Fe complex demonstrated the lowest shelf life due to the role of Fe as a potential oxidizing agent.

Keywords : Curcumin, Turmeric, Complexation, Stability, Oxidation, ASLT

1. Introduction
Turmeric (Curcuma longa L.) is a type of rhizome plant growing in tropical areas such as Indonesia. It is commonly used as food coloring or flavoring due to curcuminoid content (3-15%) and essential oil (1.5-5%). There are three types of curcuminoid content which are curcumin (85-89%), demethoxycurcumin (10%), and bisdemethoxycurcumin (1-5%) [1]. Unfortunately these polyphenol content are susceptible to degradation due to light and heat oxidation. It exhibit strong light absorbing properties and degradation when subjected to sunlight or indoor lamp. Curcumin will be degraded if exposed to 350 nm light, forming degradation products such as Z and E isomer from 3-(3,5-dimethoxyphenyl) and propenoic acid [2]. Decrease of curcumin content up to 53% also happened in food product treated with heat and pressure for 10 minutes forming vanillin, feruloyl methane, and p-hydroxybenzoic acid [3].

Stability or product shelf life can be defined as a period when a product still have acceptable organoleptic or safety quality. For curcumin as natural colorant, degradation can be marked by dulling or decrease in coloring ability causing consumers to increase the dosage and resulting in increased cost. One solution to overcome this problem is by using metal complexation (chelation) since curcumin is a bidentate ligand and can form stable complex with metal. Chelation is defined as a complex formed by several coordination bond with two or more donor atoms between organic molecules and transition metal ion [4]. Metal ions such as zinc (Zn) and magnesium (Mg) are able to increase curcumin stability up to 20 times at 37°C compared to curcumin alone. Specifically, curcumin
is considered as β-diketon and keto-enol ligand that can form coordination bond between oxygen atom and metal ions. In its free form, curcumin is easily oxidized and undergo structural change but curcumin complex can hold rectangular planar to distorted tetrahedral structural changes during oxidation [5]. Chelation itself is influenced by its chelation ring size which six ring molecule will be more stable than seven ring molecule because there is no excessive structural stress. Also, increase in temperature will decrease complex bond stability therefore the usage of complexing agent in higher temperature may lessen its effectiveness [6].

Indirect shelf life determination method to assess curcumin stability can be done by using Accelerated Shelf Life Testing (ASLT) that use Arrhenius equation and extreme condition to determine product shelf life [7]. When using this method, samples are stored in extreme condition such as hot (usually there will be three different temperature used) and humid environment to accelerate biochemical degradation resulting in quicker assessment compared to conventional method [8]. The product degradation parameter, for example curcumin content are then analyzed at certain intervals and calculated using Arrhenius equation.

2. Materials and Methods

The equipments used in this research included rotary evaporator, oven, analytical balance, glass bottle, beaker, spectrophotometer, and hotplate stirrer. Materials used in this research are turmeric powder, ethyl acetate, n-hexane, polysorbate, propylene glycol, ethanol (96%), ZnSO4 (s), MgSO4 (s), and FeSO4 (s). This research was done at PT. Bukit Warna Abadi, Ruko Sentra Niaga Bulevar Hijau kav 7-9, Harapan Indah, Bekasi from March to June 2021.

2.1. Curcumin Extraction

Turmeric powder originated from Aceh Province, Indonesia was first extracted using n-hexane solvent to separate its essential oil content. This powder was macerated and stirred for 24 h prior to filtration and oven-drying at 35°C for 72 h. Dried turmeric powder was then macerated again using ethyl acetate solvent with 1:7 turmeric:solvent ratio for 17 h continued by stirring for 6 h. Curcumin and solvent solution were filtered and separated using rotary evaporator with 40°C and -0.09 MPa waterbath temperature and pressure. Thick curcumin liquid was obtained and further dried at 35°C for a few days until its weight decrease was smaller than 0.1 gram [9]. Curcumin content analysis was done by dissolving 0.1 g curcumin in 250 ml ethanol, 1 ml of solution then transferred and diluted in 100 ml volumetric flask. Absorbance of this solution was measured at 425 and the curcumin content was calculated using equation (1). This analysis was done in triplicate [10].

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\% \text{ curcumin} = \frac{\text{Abs} \times 25000}{0.1 \times 1607} \quad (1)
\]

2.2. Sample Preparation

According to previous analysis result, calculation was done to determine sample composition which the curcumin powder was mixed with propylene glycol and polysorbate to make 11% ± 0.5% liquid curcumin (Table 1). These ingredients were mixed using stirrer for 6-8 h and covered in aluminum foil to prevent light oxidation.

| Curcumin Powder | Polysorbate | Propylene Glycol | Final Sample Weight |
|-----------------|-------------|------------------|---------------------|
| 39.87 g         | 140.13 g    | 20.00 g          | 174.20 g           |

Liquid curcumin were then divided into four containers, one container for control and another were mixed with complexing agent in 1:2 stoichiometric ratio (Table 2) [11]. For the control sample, it was divided into nine bottles which every three samples were for one storage condition. The same treatment also applied to another samples resulting in total of 36 bottles.
### Table 2. Amount of complexing agent added

|                | Control | ZnSO₄ | MgSO₄ | FeSO₄ |
|----------------|---------|-------|-------|-------|
| Liquid curcumin| 43.55 g | 43.55 g | 43.55 g | 43.55 g |
| Ratio          | -       | 1:2   | 1:2   | 1:2   |
| Complexing agent | -     | 3.30 g | 2.83 g | 3.20 g |

2.3. **Shelf Life Determination**
ASLT method was used to determine curcumin shelf life. In this case, we used three different temperature (35, 45, and 55°C). A little bit of curcumin liquid was taken every 72 hours from every bottles and stored at -20°C until all the bottles were treated. Curcumin content analysis were done again by dissolving 10 mg of sample in in 250 ml ethanol, 1 ml of solution then transferred and diluted in 100 ml volumetric flask. Absorbance of this solution was measured at 425 nm wavelength and curcumin content calculated using equation (1). This analysis was done in triplicate [10]. Curcumin concentration data was then calculated to determine its reaction order by plotting against regression curve. From that data, regression curve between temperature and pre-exponential constant was also made to obtain degradation constant used to calculate its shelf life.

3. **Results and Discussion**
In this research, powdered curcumin was extracted using two types of solvent. Normal hexane was capable to isolate essential oil content in curcumin, increasing the purity of curcumin and ethyl acetate was used instead of ethanol due to halal aspect of this product. After extraction, we recovered 5.69% of curcumin powder with 55.17% purity (>50%) meaning that these results comply with company standards. Addition of polysorbate in the product work as an emulsifier and curcumin solvent, with propylene glycol that used to increase emulsion quality (co-surfactant) and also as a preservative [12]. Every samples for each treatment were then added with complexing agent with different amount based on stoichiometric calculation to in order to form curcumin metal complex (1:2). Hence, metal with heavier molecular weight will be used more than the lighter one. This ratio and metal types were chosen with safety consideration and it might have better efficiency because single metal molecule can attract two curcumin molecules simultaneously [5]. Spectrophotometric method to determine curcumin content was used because of better accuracy and did not have expensive cost. When curcumin was degraded it will form certain transparent byproducts and decreasing its absorbance.

3.1. **Liquid Curcumin Shelf Life**
According to regression curve graph (Figure 1-4) this reaction followed zero order reaction that are commonly found in oxidation reaction. This is shown by higher R² value at the zero order graph and it means that the quality degradation occurred constantly overtime.

![Figure 1. Zero (a) and first (b) order graph of control sample](image-url)
Arrhenius curve between temperature (1/T) and ln k were done to obtain Arrhenius equation and activation energy value (Ea). It was shown that the Ea value ranged between 19-43 kJ/mol K (Table 4) and considered as low Ea while theoretically, Ea value can ranged between 2-400 kJ/mol [13]. Reactions including oxidation can be easily occurred in lower Ea value so lower Ea may result in shorter product shelf life. But in this case, the results were reversed for example control sample with Ea 43.38 kJ/mol K had shorter shelf life than curcumin-Zn sample with Ea 19.87 kJ/mol K. There is possibility of Ea changes during reaction due to light or air exposure therefore, lower Ea value does not represent higher reaction rate or it can be stated that reaction can occur slowly during initial period but the rate will increase overtime and vice versa.
Table 3. Activation Energy Data

| Temperature (°C) | 1/T   | k₀    | ln k  | Ea (kJ/mol K) |
|------------------|-------|-------|-------|---------------|
| Control          |       |       |       |               |
| 35 (308 K)       | 0.003247 | 0.0191| -3.9581|              |
| 45 (318 K)       | 0.003145 | 0.0315| -3.4577| 43.383        |
| 55 (328 K)       | 0.003049 | 0.0537| -2.9243|              |
| Curcumin-Mg complex |     |       |       |               |
| 35 (308 K)       | 0.003247 | 0.0191| -3.9581|              |
| 45 (318 K)       | 0.003145 | 0.0228| -3.7810| 26.945        |
| 55 (328 K)       | 0.003049 | 0.0364| -3.3132|              |
| Curcumin-Zn complex |     |       |       |               |
| 35 (308 K)       | 0.003247 | 0.0173| -4.0570|              |
| 45 (318 K)       | 0.003145 | 0.0210| -3.8632| 19.875        |
| 55 (328 K)       | 0.003049 | 0.0278| -3.5827|              |
| Curcumin-Fe complex |     |       |       |               |
| 35 (308 K)       | 0.003247 | 0.0210| -3.8632|              |
| 45 (318 K)       | 0.003145 | 0.0352| -3.3467| 25.418        |
| 55 (328 K)       | 0.003049 | 0.0383| -3.2623|              |

Reaction rate constant from Arrhenius graph was plotted against the formula resulting in final product shelf life. From Table 4, we know that curcumin-Zn complex have higher shelf life at 35°C compared to control and curcumin-Mg complex while curcumin-Fe have the lowest shelf life. Calculation was also done for 25°C since it is better to store the product at lower temperature and protected from light to prevent degradation. But at 25°C control sample become more stable than another. It might be caused by sub-Arrhenius curve type that formed in this research. This means there is possibility of higher reaction rate at lower temperature than expected. After certain point, the shelf life value can be different from expected [14]. This data also obtained from calculation only and not using actual analysis which might affect its accuracy so it need further research.

Table 4. Product shelf life

| Types  | 25°C | 35°C | 45°C |
|--------|------|------|------|
| Control | 242.7| 137.5| 80.7 |
| Mg     | 202.9| 142.6| 94.5 |
| Zn     | 193.5| 152.2| 110.0|
| Fe     | 163.0| 116.9| 85.5 |

3.2. Complexing Agents Effectivity

It can be stated that complexing curcumin with metal ions using 1:2 ratio can improve its stability and shelf life at 35°C up to 10-15 days except for curcumin-Fe complex. This phenomenon are related to curcumin degradation rate which affected by chemical properties of each complexing agents. Control sample have lower shelf life because curcumin molecules are not bound into complexes and easily degraded when subjected to high temperature. Whereas curcumin-Mg and curcumin-Zn complexes have longer shelf life because their diketone or keto-enol moiety have coordination bond with metal ions, increasing their stability.

Several factors that affect each complexing agent characteristics are metal ion properties, ligand, and macrocyclic effect which cause Zn metal to be highly effective in forming a complex. Metal ion properties including oxidation number and ionic radius size can affect complex strength although all of the reagents have same oxidation numbr (+2) but Zn have smaller ionic radius size (135 pm) meaning it have higher electostatic energy compared to larger radius in Mg (150 pm). A previous study also
stated the distance between coordination bond in curcumin-Zn were 1.99 and 1.98Å, 1.98 and 1.96Å, 2.18 and 2.16Å, 2.25 and 2.22Å, curcumin-Fe 2.01 and 1.99Å, 1.97 and 1.93Å, 2.27 and 2.09Å, 2.27 and 2.26Å, curcumin-Mn 2.01 and 2.00Å, 2.02 and 1.99Å, 2.26 and 2.21Å, 2.32 and 2.26Å. So curcumin-Zn complex is more stable because of smaller ionic radius. Related into its binding affinity, curcumin-Zn exhibited higher interaction energy value (-624.51 compared to -620.51 and -597.35 kcal/mol for Mn and Fe [15]). Another theory stated that metal with less number of unpaired electron have shorter coordination bond distance. In this case, Fe have four unpaired electron while Mg and Zn does not have any [16]. This also cause Fe to have higher oxidizing properties since it must attract other electron to become stable. Decreasing Fe metal dosage into 1:3 ratio might decrease its reactivity and form stable complex with curcumin [11]. Although there were no previous studies regarding curcumin complex stability in food coloring products, studies regarding other application such as pharmaceutical prove that pure curcumin alone was degraded totally in one hour at neutral pH buffer, conversely degradation rate of curcumin-Zn complex decreased to 95% in the same condition [17].

3.3. Other Factors
Apart from the theory about each reagents chemical properties causing curcumin-Fe to have lowest stability. There are another factor affecting this condition. For example, when maximum absorbance wavelength was scanned using spectrophotometer, maximum wavelength shifting was occurred from 425 to 416 nm. This can cause error in calculation making it have more or less curcumin content than the true value since the formula is designed to calculate curcumin content at 425 nm. Visually, dissolved sample in ethanol (Figure 5) also have significant difference from the others and it have brownish hue because of FeSO₄ reagent that have blue color originally.

![Figure 5. Samples color appearance](image)

Overall result from this study have not fulfill the target which expect one year of product shelf life in room temperature. Compared to other natural coloring products for example, beta carotene, curcumin, and carbon black they have longer shelf life (1-5 years). Opening and recapping sample bottles repeatedly for 18 days allows light and oxygen to came into contact with curcumin making oxidation to occur much faster compared to conventional method using in existing product assessment which sample observation are done in longer interval. Another consideration is the usage of reagent types or formulation are not suitable enough for this application. For example in a study done by Sareen et al., [18] they used ZnCl reagent with 1:1 ratio. Similarly with study done by Prasad et al., [19] also used 1:1 ratio with water addition. The usage of higher dose and water may increase curcumin molecules stability and better solubility of the metal ions resulting in better complexation reaction.

4. Conclusion
Curcumin metal complexing can increase liquid curcumin stability for 10-15 days at 35°C compared to curcumin alone with longest shelf life exhibited by curcumin-Zn complex (152 days) followed by curcumin-Mg (142 days) and control (137 days). This is caused by complexation reaction that prevents curcumin degradation. Curcumin-Fe complex have the lowest stability and shelf life (116
days) due to the stronger oxidizing properties of Fe, thus leading to curcumin oxidation instead of complexation.

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