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Determination of Antioxidant Activity and Identification of Organophosphate Pesticide Residues on Red Chili Pepper (*Capsicum frutescens* L.)

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Abstract

This study assessed the effects of storage duration on the antioxidant activity and the presence of organophosphate pesticide residues in red chili peppers (*Capsicum frutescens* L.). The research employed the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to measure antioxidant activity and a colorimeter-based method for detecting pesticide residues. Red chili peppers from Bandung, Indonesia, were analyzed independently for these parameters. The analysis revealed detectable organophosphate residues in chilies from Market B, whereas those from Market A were free of residues. Antioxidant activity, evaluated over storage periods at room temperature (28°C), remained above 200 ppm, with IC50 values of 607.87 µg/mL, 551.25 µg/mL, and 713.56 µg/mL after 5, 10, and 15 days, respectively. While pesticide levels were within safety limits, a notable decrease in antioxidant activity was observed, attributed to reductions in vitamin C, capsaicin, and phenolic compounds.

Keywords: *Antioxidant Activity, DPPH Assay, Organophosphate Pesticides, Red Chili Pepper*

1. Introduction

Cabai rawit or red chili pepper (*Capsicum frutescens* L.) is a horticultural commodity closely intertwined with the daily lives of the Indonesian people. It is generally utilized as a cooking spice or flavor enhancer. According to the National Socioeconomic Survey (Susenas) conducted in September 2021, the average consumption of red chili peppers among Indonesians was recorded at 0.04 kg per capita per month. Statistical data indicated that the production of red chili in Indonesia had increased over the past five years reaching 1.5 million tons in 2022.

In addition to its culinary uses, red chili peppers offer health benefits due to their antioxidant properties. Some compounds in red chili which have antioxidant potential include vitamin A, vitamin C, flavonoids, capsaicinoids, phenolics, and carotene (1). Several studies have indicated that storage conditions affect the stability of these compounds in chili (2–5).

Despite these advantages, chili farmers frequently face challenges with their crops, such as pests and diseases, leading to harvest failures and financial losses. Diseases affecting chili plants include leaf spots, fruit rot, yellow virus, and leaf curl virus (6). To mitigate these losses, farmers often resort to using pesticides on their produce, without considering the potential health hazards of such overuse.

Pesticides are substances or mixtures specifically used to control, prevent, or repel insects, rodents, viruses, bacteria, and other organisms deemed as pests. Farmers strive to manage pests to prevent them from attacking red chili plants. The high usage of pesticides among farmers is attributed to their effectiveness in pest control and ease of use. The harvested red chili is then distributed to markets, where it will be purchased by consumers. Food safety is crucial as it impacts health, national trade, and global markets. Consumer demands for food safety are also essential for enhancing the competitiveness of fresh plant-based commodities export markets (7).

We conducted separate analyses on red chili peppers to detect their organophosphate pesticide residues and assess their antioxidant properties during storage. It is important to note that these analyses were performed independently of each other, each addressing distinct aspects of the red chili pepper's safety profile and composition.

2. Materials and Methods

2.1 Materials

The red chili peppers (*Capsicum frutescens* L.) were obtained from Bandung, Jawa Barat, Indonesia.

2.2 Detection of Organophosphate Pesticide Residues

The pesticide detection kit used was based on colorimetric from Chemtest. The sample was cut into small pieces, then treated with washing solution with a sample to washing solution ratio of 1:2. The protective layer of the pesticide detection card was removed, and 2-3 drops of sample water were applied onto the white circle, followed by a 5 minutes incubation period. Subsequently, the card was folded in half and pressed for 3 minutes, allowing the white and red disks to react until a color change occurred in the white circle.

2.3 Analysis of Antioxidant Activity

2.3.1 Sample Preparation

Red chili peppers were weighed, sorted, and stored for 5, 10, and 15 days at room temperature. After the storage period, they were separated from stems, washed, and crushed. The chilies were extracted using 96% ethanol (1:20 ratio) and macerated for 3 days. The filtrate was then evaporated using a rotary vacuum evaporator at 45^{o}C for 2-4 hours until thickened.

2.3.2 DPPH Solution Preparation

A stock solution of DPPH was prepared at a concentration of 160 ppm. DPPH was weighed and dissolved in chemical analysis grade methanol. The DPPH solution was homogenized using a stirrer. Subsequently, the solution was transferred into a dark 25 ml volumetric flask and adjusted to the mark with methanol.

2.3.2 Determination of Antioxidant Activity

This procedure was adapted from earlier study to conduct antioxidant analysis in this study (8). The chili extract was dissolved in methanol and homogenized using a stirrer. A blank was prepared by mixing 2 mL of methanol with 0.5 mL of DPPH stock solution. The solutions were then diluted to various concentrations (1000, 500, 250, 125, 62.5 ppm) by mixing sample, DPPH stock solution, and methanol to a total volume of 2.5 mL each. Each mixture was incubated in the dark for 30 minutes at room temperature. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

3. Results and Discussion

3.1 Organophosphate Pesticides Residues

During chili production, farmers often encounter challenges such as pest attacks and diseases, which can significantly reduce or even cause crop failures. Pest infestations lead to substantial financial losses. Continuous use of pesticides can also lead to environmental pollution and leave residues on agricultural products, posing health risks to consumers when consumed.

Based on the identification results using the pesticide detection card, red chili peppers from Market A were not detected to contain organophosphate pesticides. This is indicated by the lack of color change in the white circle of the Pesticide Detection Card after testing, indicating a negative result for pesticide residues. For red chili peppers from Market B, although organophosphate pesticides were detected, the concentration was relatively low.

The pesticide detection card uses chromogenic reagent and cholinesterase technology to rapidly detect residues of organophosphates and carbamates commonly used in vegetables and fruits. This kit provides qualitative results, indicating only the presence or absence of pesticide residues without quantification (9).

In this test, if the sample contains pesticides, the pesticide detection card will show a color change in the white circle as an indicator. A positive result is indicated by no color change, while a change to light blue indicates low pesticide levels. In the case of chili from Market A, there was no color change, confirming that the chili is safe for consumption without significant pesticide residues.

Fig. 1 shows change of color on the detection card caused by chili from Market A and Market B

The results obtained from the study indicated that the chili from Market B was detected to contain organophosphate pesticides. This is based on the color change observed on the pesticide detection card, which turned a faint blue, indicating that it tested positive for pesticides, although the levels were not very high. Washing with food-grade liquid detergent is considered effective in reducing pesticide residue on vegetables and fruits, as the detergent can dissolve pesticides that are not water-soluble. The results for each type of chili indicate that the chilies are still safe to consume because the pesticide residue levels detected by the rapid test kit are still within the safe limits for pesticides. However, this still needs to be noted and monitored, as even low levels can still have adverse effects on health.

Table 1: Detection results of organophosphate pesticide residues in red chili peppers

Sampling Location	**Result**
Market A	Undetected
Market B	Detected

Organophosphates are known as the most toxic insecticides among other pesticides. The organophosphate group constitutes the largest number of pesticides circulating in the market and is widely used in agriculture (10). The presence of pesticide residues is generally influenced by factors such as the type of pesticide, the dosage used during application, the method of pesticide application, weather, and climate conditions such as rain, which can reduce the level of pesticide residues remaining in the environment (7) Using the appropriate pesticide dosage can result in relatively low residue concentrations (11). Additionally, the degradation of pesticides is influenced by several factors, including application factors (time, speed, application position, etc.), pesticide properties (toxicity, persistence, volatility, etc.), microorganisms, and the timing and speed of application which cause pesticide loss (12).

Organophosphate pesticides degrade easily and are highly volatile. Degradation for all types of pesticides is on average >80% within 10 days after application (13). Organophosphate pesticides do not take long to evaporate. Pesticides that have evaporated into the air will break down under the influence of temperature, humidity, and sunlight, particularly ultraviolet rays. This decomposition process occurs gradually (14). Farmers stop spraying pesticides on chili plants one week before harvest. This is done to ensure that the harvested produce does not contain excessive pesticide residues and does not exceed the permitted limits (15).

During the distribution from farmers to sellers in the market, pesticide degradation on chilies can occur. Additionally, surface residues can disappear due to washing and hydrolysis. If it rains when plants are treated with pesticides, it's likely that pesticide residues have been washed away, and the remaining residues degrade under ultraviolet light (12). The reduction or disappearance of residues on chilies can be caused by evaporation during the distribution process from farmers to the market. Evaporation can occur because organophosphate pesticides do not take long to evaporate. Temperature and wind speed can affect the rate of pesticide residue degradation. Weather and climate in highland areas tend to favor plant pests, so pesticide use in highland areas is more intensive compared to lowland areas.

3.2 Antioxidant Activity

Antioxidant activity is defined as the ability of a compound or extract to inhibit oxidation reactions, which can be expressed as a percentage of inhibition. One of the parameters used to indicate antioxidant activity is the Inhibition Concentration (IC50) value. IC50 is the concentration of an antioxidant substance that gives 50% inhibition. Substances with high antioxidant activity will produce low IC50 values (16). IC50 values less than 50 µg/mL are considered very strong, 50-100 µg/mL are strong, 100-150 µg/mL are moderate, 150-200 µg/mL are weak, and more than 200 µg/mL are very weak (8).

DPPH, or 1,1-diphenyl-2-picrylhydrazyl, is a stable free radical compound. The principle of measuring antioxidant activity using the DPPH method is based on observing the fading of the purple color of the DPPH radical due to the presence of antioxidants that can neutralize free radical molecules by hydrogen atom donation to DPPH. The purple-colored DPPH solution, when encountering electron-donating substances, undergoes reduction, causing the purple color to fade and change to yellow derived from the picryl group (17). The DPPH testing method is advantageous because DPPH can remain stable for years under dry storage conditions with proper handling. The maximum absorption of DPPH occurs at a wavelength of 517 nm (8).

One of the roles of antioxidants is to neutralize free radicals by inhibiting lipid oxidation generated by free radicals. The stable free radical DPPH has been widely used to test various plants rich in antioxidants. Fresh Capsicum frutescens shows strong antioxidant activity due to the presence of a number of phenolic compounds, flavonoids, and proanthocyanidins (18).

Table 2: Antioxidant activity of red chili peppers at room temperature storage

Storage Time (Days)	IC_{50} Value (*µg/mL)*
5	607,87 ± 0,01
10	551,25 ± 0,01
15	713,56 ± 0,01

The test results (Table 1) of storage at room temperature showed significantly different antioxidant activity values. Red chili pepper at each storage period at room temperature (28°C) exhibited antioxidant activity above 200 ppm, indicating very weak activity. The testing results using the DPPH method are expressed in IC50 parameter (inhibition concentration 50). The IC50 values of red chili pepper after storage for 5, 10, and 15 days, respectively, were 607.87 µg/mL, 551.25 µg/mL, and 713.56 µg/mL.

The antioxidant activity testing using the DPPH method on fresh Capsicum frutescens fruit showed strong antioxidant activity with an IC50 value of 2,751 µg/mL (19). Several factors influencing antioxidant activity include genetics, environmental conditions such as temperature, and physiological factors (20). Storage of red chili pepper at 30°C showed a decrease in capsaicin content, which is one of the antioxidant compounds in red chili pepper (2). Other antioxidant compounds such as vitamin C and phenolics also showed a decrease during storage (4,21). Optimal storage temperature to maintain the quality of red chili pepper is between 4-10°C (22).

4. Conclusions

In conclusion, the analysis of *Capsicum frutescens* L. confirms their safety for consumption, as indicated by pesticide residue levels within safe limits detected by rapid test kits. Furthermore, the research findings demonstrate that the antioxidant activity of red chili peppers stored at room temperature is significantly weakened. This decline in antioxidant capability is primarily due to reductions in vitamin C, capsaicin, and phenolic compounds, all crucial antioxidants in red chili peppers.

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