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Physical, microbial and pesticide contaminations on fresh vegetable and fruit marketed in Samarinda-Indonesia

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Abstract. Fresh vegetables and fruits are a source of antioxidants. However, the hazard carried in the form of physical, microbial, and pesticide contaminations may limit the benefit of consuming the fresh produce. This research aims to measure the antioxidant content of fresh vegetables and fruits in traditional and modern markets in Samarinda, Indonesia, as well as the physical, microbial, and chemical contamination of the respective samples. Ten vegetables and fruits were representing the fresh food market, namely dragon fruit, asparagus, strawberry, golden kiwi, beetroot, cauliflower, green mustard, lemon, paprika, and purple sweet potato. A cross-sectional sampling method carried out in February 2019. Physical or environmental contaminations were observed on the market shelves. Microbial, pesticide contamination, and antioxidant content of the vegetables and fruits were directly measured on the day of purchase. Antioxidant substances were marked by total phenolic, alpha-tocopherol, and ascorbic acid. The vegetable and fruit sold in Samarinda-Indonesia contained relatively high antioxidant, indicated by total phenolic, alpha-tocopherol, and ascorbic acid. However, the above limit cypermethrin was detected in strawberry, golden kiwi, and lemon. Physical quality of vegetables and fruits may lead to microbial contamination. Of the selected vegetables and fruits, microbial contamination remained a significant hazard, followed by pesticides.

1. Introduction

Fresh vegetables and fruits are a source of antioxidants. Freshness of vegetables and fruit can be deduced by the amount of total phenolic content and antioxidant capacity [1]. The activity of antioxidants from vegetables and fruits can be measured through singlet oxygen reduction and hydrogen atom transfer [2]. While variety, climate, harvesting season, and soil may influence the fluctuation of phenolic thus anti-oxidative strength of the vegetable and fruits, the model of relating vegetable freshness can be developed from the quantification of these parameters [3]

On the other hand, the serious challenges of serving fresh vegetables and fruits are the microbial and chemical contaminations obtained during planting, harvesting, and minimally processing of the intended vegetable and fruits [4, 5]. Fresh vegetables and fruits are in growing demand in emerging modern markets like Indonesia and other Southeast Asian countries [6].

Irrespective of the availability of technical guidelines, counseling, and constant improvement by the government and more stringent requirements set by the modern food market [7], the challenge of serving less microbial and chemically harmful vegetable and fruits exists in Indonesian market. Global surveillance of fresh produce has been reported [8]. However, in emerging modern cities of Indonesia, i.e. Samarinda, the surveillance is rarely documented. Moreover, the surveillance report is often presented only the hazard observed. This paper try to highlight not only the hazard in fresh vegetables and fruits, but also the antioxidant advantage of the respective samples.

This research aims to measure the physical, microbial, and chemical contamination of fresh vegetables and fruits in the modern market in Samarinda, Indonesia, as well as the antioxidant contents marked by the phenolic, ascorbic acid, and alpha-tocopherol.

2. Experimental methods

Samples of fruit and vegetables were aseptically obtained from two markets in Samarinda, Indonesia, namely Pasar Inpres Baqa, located at Gg. Langgar, Baqa, Samarinda Seberang, Samarinda, East Kalimantan, and Lotte Grosir located at Jalan HM Jl. Kadrie Oening, Air Hitam, Samarinda Ulu, Samarinda, East Kalimantan. Ten vegetables and fruits were representing the fresh food market, namely dragon fruit, asparagus, strawberry, golden kiwi, beetroot, cauliflower, green mustard, lemon, paprika, and purple sweet potato. The samples were obtained in a cross-sectional method in February 2019. Physical contaminations were observed on the market shelves. Microbial, chemical (pesticide) contamination, and antioxidant content of the vegetables and fruits were directly measured on the day of purchase.

2.1. Physical contamination and general observation

Physical contamination and general inspection were carried out visually, and the results were recorded. The observations include dirt, dust, water, condition of container, shelves temperature, outer texture, fungal infestation, and injury.

2.2. Microbial contamination

The quantitative method for calculating microbial contamination is the Total Plate Number (TPC) and Total Yeast and Fungi (TYF). TPC is used to calculate the rate of contamination of mesophyll aerobic bacteria found in the sample. A total of 1 mL of serially diluted samples (from 10^{-2} to 10^{-5}) were poured on solid media of Nutrient Agar (Oxoid, USA). Petri dishes were then incubated for 24–48 hours at 37°C in the upside-down position. TYF is used to calculate yeast and fungi contamination contained in the sample. One mL of serially diluted samples (from 10^{-2} to 10^{-5}) was poured into Dichloran Rose Bengal Agar (DRBC) solid media (Oxoid, USA). Petri dishes were then incubated at room temperature (28±3°C) for 72–96 hours.

2.3. Pesticide contamination

Pesticide contamination was detected qualitatively before quantitative measurement was accomplished. G9 Fast Pesticide detection (PT Purnama Laboratory, Indonesia) was used to identify residual pesticide. About 10 g of samples were carefully blended and eluted with distilled water until vegetable or fruit juice was formed. The juice was dropped on the detection holes. If one of the hole color indicators giving diminishing blue to white color, then the test for the particular vegetable or fruit was continued with a quantitative method. The residue of pesticide was spectrophotometrically quantitated (BioSpectrometer®, Eppendorf, Germany), employing cypermethrin (PT Bayer, Indonesia) as pesticide standard. About 25 grams of samples were added with 100 mL of ethanol (Fulltime, China) and then blended for two minutes or until a homogeneous slurry was formed. Then the sample was filtered, and the pulp was rinsed twice with 25 mL of ethanol. The filtrate is collected and evaporated in batches at a temperature of 50°C until the volume produced was approximately 20 mL. This sample solution was used for quantification. The optimum wavelength of cypermethrin was determined by scanning. The optimum wavelength was obtained at 420 nm. The standard curve was obtained by serial measurement of cypermethrin (0.1 to 0.5 ppm).

2.4. Total phenols
Total phenols were measured by modifying methods initially developed in previous research [9, 10]. Whole fruit and vegetable were blended and macerated for 24 h in absolute ethanol (Fulltime, China) at 1:3 or 1:4 ratios. The filtrates were then oven-dried at 50±5°C. As much as 0.3 g of dried ethanolic extract of each vegetable or fruit was carefully weighed and dissolved in 10 mL of ethanol (Fulltime, China) and distilled water (Soil Laboratory, Mulawarman University) at ratio of 1:1. About 0.2 mL of the extract solution was taken and added with 15.8 mL of distilled water and 1 mL of 50% (v/v) of Folin-Ciocalteu (Sigma-Aldrich, Germany) reagents prepared in aqueous solution. The solution was allowed to stand for ±8 minutes and added with 3 mL of 5% (w/v) of Na_2CO_3 (Sigma-Aldrich, Germany) prepared in aqueous solution. The solution was further allowed to stand for ±2 hours in a dark condition at room temperature (28±2 °C). The absorbance was measured at 725 nm of wavelength. The absorbance was plotted against the standard curve of Gallic acid prepared in the same manner. Total phenols were expressed in mg equivalent of Gallic acid per kg of dry weight.

2.5. Ascorbic acid
Vitamin C contents were measured according to a previously established method [11]. Standard and chemicals were purchased from Sigma-Aldrich (USA).

2.6. Alpha-tocopherol
Whole fruit and vegetable were blended and macerated for 24 h in absolute ethanol (Fulltime, China) at 1:3 or 1:4 ratios. The filtrates were then oven-dried at 50±5°C. As much as 0.3 g of dried ethanolic extract of each vegetable or fruit was carefully weighed and dissolved in 10 mL of ethanol (Fulltime, China). The determination of α-tocopherol was accomplished by interpolating the absorbance of the sample with a standard calibration curve of α-tocopherol at a wavelength of 291 nm (BioSpectroMeter®, Eppendorf, Germany). The α-tocopherol standard (Sigma-Aldrich, UK) was prepared in absolute ethanol (Smartlab, Indonesia) with various concentrations of 50, 90, 120, 160, and 200 mg/L in 10 mL of absolute ethanol. The blank sample was prepared without containing the active substance of α-tocopherol.

3. Results and discussion

3.1. Physical contaminations and general observation
Ten vegetables and fruits were carefully selected to represent the condition of fresh produce sold in Samarinda-Indonesia. In general, physical contamination of fresh food exists regardless of the origin of the crop (table 1). Transportation and on shelve handling of the vegetable and fruits require better practice so that the consumer can receive better quality of produce from local markets. Educating suppliers play a crucial role in succeeding effort. The producer and supplier should have a better understanding of quality, thus develop a practical approach to manage quality and handling of fresh produce. In the end, an integrated effort will ensure the safety of fresh fruits and vegetables from various threats, such as physical contaminants [12]. Integrating good practice on the handling of fresh produce will reduce the burden of produce decontamination from pathogenic threats, thus contributing to better protection of consumer health [13].

Table 1. Physical contamination and general observation.

Vegetable/Fruit name	Physical contamination and general observation
Dragon fruit	dust, rainwater, uncleaned wooden container
Asparagus	not wrapped, easily being cross-contaminated
Strawberry	fluctuated shelves temperature: -25.0 to 10.4 C.
Golden Kiwi	Soft texture, almost rotten
Beetroot	visible injury during packaging
Cauliflower	Leaf are partially damaged
Green Mustard	dust, rainwater, uncleaned wooden container
Lemon	soft texture
Paprika	soft texture
Purple sweet potato	visible soil dirt

3.2. Microbial contamination

Currently, no legal limits of TPC and TYF on fresh vegetables and fruits established within SNI 7388:2009 and Regulation of the Head of the Food and Drug Supervisory Agency (Perka BPOM) 16/2016. However, it is necessary to address that potential pathogenic microbes may increase in high count of total plate count and total yeast and fungi (table 2). For example, Salmonella, E. coli, and mycotoxigenic fungi may infect vegetables or fruit as a result of soil and unclean water exposure, or poor handling practice since the pre-harvest stage. The importance of pre-and postharvest handling to reduce the initial microbial contaminants, thus reducing the safety hazards of fresh produce [14]. In place of the statement, the integrity of clean produce handlers are a solution to control safety hazard caused by pathogenic microbes in fresh produce [15].

Table 2. Microbial contamination.

Vegetable/Fruit name	TPC (CFU/g)*	TYF (CFU/g)*
Dragon fruit	3.7×10^6	9.7×10^4
Asparagus	4.1×10^6	4.3×10^5
Strawberry	8.0×10^5	2.5×10^4
Golden Kiwi	1.8×10^6	3.6×10^4
Beetroot	4.3×10^6	3.0×10^5
Cauliflower	2.5×10^6	5.5×10^6
Green Mustard	5.3×10^6	3.7×10^6
Lemon	1.2×10^3	1.5×10^5
Paprika	2.7×10^6	2.8×10^5
Purple sweet potato	5.0×10^6	4.7×10^6

*Limits of TPC and TYF on fresh vegetables and fruits are not established within SNI 7388:2009 and Regulation of the Head of the Food and Drug Supervisory Agency (Perka BPOM) 16/2016.

3.3. Pesticide contamination

From the qualitative test, strawberry, golden kiwi, and lemon were not passed the pesticide detection. This indicated that in any stage before the fresh produce is available on the shelf, the product may be exposed to a certain pesticide (table 3). The quantitative measurement indicated that all the fresh produce had above the maximum residual limit (MRL) of cypermethrin (table 3). According to the available standard to Joint Decree of the Minister of Agriculture and Minister of Health concerning the Maximum Limits of Pesticide Residues to Agricultural Products, cypermethrin limit is 0.05 ppm. This indication should be future confirmed with a high-performance chromatography or gas chromatography method to obtain a better resolution on the exposed pesticide. Reducing pesticide contamination in fresh produce is among the essential effort in agriculture. Since the origin of the fresh produce may be from overseas, it is required to ensure that the global food chain is operating with an integrated effort in reducing dangerous chemical hazard i.e. pesticide [16].

Table 3. Qualitative and quantitative cypermethrin detection.

Vegetable/Fruit name	Qualitative detection	Quantitative detection (ppm)	Interpretation*
Dragon fruit	Below threshold		
Asparagus	Below threshold		
Strawberry	Positive	0.10±0.02	Above MRL
Golden Kiwi	Positive	0.35±0.04	Above MRL
Beetroot	Below threshold		
Cauliflower	Below threshold		
Green Mustard	Below threshold		
Lemon	Positive	0.95±0.01	Above MRL
Paprika	Below threshold		
Purple sweet potato	Below threshold		

* cypermethrin limit is 0.05 ppm according to Joint Decree of the Minister of Agriculture and Minister of Health Number 881/Menkes/SKB/VIII/1996 771/Kpts/TP.270/8/1996 concerning the Maximum Limits of Pesticide Residues to Agricultural Products.

3.4. Total phenolic, ascorbic acid, and alpha-tocopherol

The increasing trend to consume fresh produce, i.e. vegetable and fruit, based on the growing consumer perception that fresh produce will give better nutrients and antioxidant benefits [17]. The ten vegetable and fruit sold in Samarinda-Indonesia contained relatively high phenolic and alpha-tocopherol (table 4). The goodness in the vegetable and fruit should be better protected by reducing the physical, microbial, and pesticide contaminations. Based on the focused group discussion, the degree of importance in ensuring the reduction of hazards from fresh produce are (1) good agricultural practices (GAP), (2) good hygienic practices (GHP), and (3) food safety management systems (FSMS) [8].

Table 4. Total phenolic, ascorbic acid, and alpha-tocopherol.

Vegetable/Fruit name	Total Phenolic (mg GAE/Kg dried EtOH extract)	Alpha-Tocopherol (ppm dried EtOH extract)	Ascorbic Acid (mg/100 g)
Dragon fruit	752.52±5.26	566.03±2.24	19.85±0.72
Asparagus	765.54±2.63	555.71±21.3	104.92±7.10
Strawberry	839.94±12.34	275.56±4.49	80.31±2.53
Golden Kiwi	741.36±5.26	149.63±8.74	82.35±3.97
Beetroot	614.89±21.04	322.65±0.92	43.83±0.42
Cauliflower	893.87±116.13	240.63±1.30	18.83±0.00
Green Mustard	376.82±15.78	194.07±18.40	66.45±4.16
Lemon	1343.97±15.78	380.32±22.71	86.94±2.53
Paprika	1048.25±2.63	719.21±52.64	95.48±6.04
Purple sweet potato	358.22±57.87	500.56±26.00	8.88±1.08

4. Conclusion

The ten vegetable and fruit sold in Samarinda-Indonesia contained relatively high antioxidant compounds, indicated by total phenolic, alpha-tocopherol, and ascorbic acid. However, the benefit of consuming the fresh produce may be limited by the hazard carried in the form of physical, microbial, and pesticide contaminations. Of the selected vegetable and fruit, microbial contamination remained a major hazard, followed by pesticides. Better practice from pre-to postharvest handling of fresh produce is suggested as a solution to reduce the hazards, therefore contributing to better consumer health.

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