EFFECTS OF WASHING TREATMENT ON THE CHARACTERISTICS OF ROADSIDE FRESH FRUITS

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ABSTRACT

Roadside fruits are very possible to be contaminated by heavy metals and microbial contamination, especially fruits that are usually consumed unpeeled, such as grapes, water guava, apples, and starfruit. Heavy metal contaminants that are hazardous to health include lead (Pd), cadmium (Cd), and arsenic (As), while harmful microbial contaminants include *Escherichia coli* and *Salmonella thypii*. This study aims to determine the effect of washing treatment on the characteristics (heavy metal and microbial contamination) of some fresh fruit. Washing process using running water and food grade soap. The design of the study used is Randomized Block Design, if it had a significant effect, it was further tested using the Duncan Multiple Range Test (DMRT). The results of study showed that washing with food grade soap is preferable, as it shows a significant reduction in the levels of heavy metal and microbiological contents up to 0.000 mg / kg for Pb and Cd; 0.041 mg / kg for As; 0 kol / g for E. coli and 0 kol / 25g for *Salmonella thypii*.

Keywords: food grade soap, Fruits, heavy metal, microbial contamination

INTRODUCTION

Fruits are an essential part of the human diet. They are sources of many essential nutrients, vitamins, minerals, have high water and fiber content. Fruits that can be directly consumed without needing to be peeled such as grapes, apples, water guava, star fruit, and many others. Farmers or sellers often sell fruits at roadside stands. Road side fruits are potentially exposed to exhaust gases or vehicle fumes that contain heavy metal contamination. Lead, cadmium, and arsenic are among the most risky heavy metals when consumed through contaminated fruits. Of all the heavy metals, cadmium and lead have more significant side effects on human health since they are easily accessible through food chain transmission (Rahimi, 2013). Consumption of roadside fruits also potentially increases the risk of foodborne illness caused by microbial contaminants. The common microbial contaminants are *Esceherichia coli* and *Salmonella thypii*. Another way to reduce heavy metal contamination and microbiology in roadside fruits is washing treatment. Most people wash fruits only by using water. However, this has not guaranteed the contamination of heavy metals and microbiology lost from fruits. Nowadays there are several food grade soap products that contain anionic and antibacterial agent surfactants that are safe to use to wash fresh foods.

This study aimed to measure the effectiveness of washing treatment for reduce the presence of heavy metals and microbiology contamination in road side fruits. The study was carried out using fruits that were purchased directly without washing, compared to washed fruits with running water, and washed fruits using food grade soap. The samples used for research are fruits that are usually consumed directly without peeling, such as grapes, apples, water guava, and star fruit. The results of this study are expected to provide information to the public about the dangers of heavy metals and microbiological contamination of fruits, and can educate the public to do proper washing of fruits, especially fruits that are usually consumed directly without peeling.

MATERIALS AND METHODS

Materials

This study uses four types of samples, there are red grapes, Washington apple, Semarang water guava, and Dewi starfruit. Red grapes and Washington apples represent imported fruits while Semarang guava fruit and Dewi star fruit represent local fruits. The fruits used in the study were purchased in the same place, which is in the stalls along the roadside of Pulo Jahe market, East Jakarta. The washing solution used is food grade soap. The active ingredients used in food grade soaps are anionic surfactants and anti-bacterial food grade agents that can clean pesticides and kill germs up to 99%. The concentration of washing solution used for fruit washing was also the same for each type of fruit, the formulation is 1⁄2 tbsp (7.5 ml) dissolved in 1 liter of water.

Materials used for testing include, aquadest, acidic solution, Pb standard solution; CD; and As, Modified Letheen Broth media; Nutrient Agar and Sabuoraud Dextrose Agar; Cromocult Agar; and Xylose Lysine Deoxycholate. The tools used for testing included a basin, hollow and plastic basin, beaker glass, stirring rod, hot plate, measuring flask, glass funnel, filter paper, Atomic Absorption Spectrophotometry (AAS), Gas Chromatography (GC), magnetic stirrer, bottles sterile, test tube, finnpipette, pipette tips, autoclave, Bio Safety Cabinet (BSC), and incubator.

Procedure

The steps of determining the effect of washing on heavy metal and microbiological contamination on fresh fruit can be seen in Figure 1 and Figure 2. In the first step, the fruits analyzed were selected through screening results including the selection of all types of fruits that can be consumed without peeling the skin that are sold in fruit stalls along the Pulo Jahe market road using descriptive methods. The microbiology test carried out was the total calculation of microbes including bacteria, fungi, and yeast using the Total Plate Count (TPC) method. While the chemical test using the Gas Chromatography (GC) method.

Chemical Test

Heavy Metal Contents

Fruit samples prepared before reading with AAS instrument. The fruits were initially deconstructed, which was weighed as much as 100 grams in a porcelain dish, burned, and ashed in a furnace at 900°C for 1 hour. Then the ash filtrate was acidified with the addition of 1 ml of 1: 1 HCl and being heated. After dissolving, put the solution into a 100 ml measuring flask and add aquadest to the boundary mark. The solution is filtered and ready to read the content of heavy metals with the AAS instrument at the wavelength specified for each heavy metal.

Moisture Content (AOAC, 2006)

Weighed each dish that will be used. weighed ± 5 grams of sample into the dish, and put the dish in the oven at 105°C for ± 4 hours or until the weight is constant, then cooled in the desiccator, and weighed at the final dish with its sample. The calculation of wet and dry base water content was used. The formula for calculating water content is as follows:

Moisture content (wet base) (%) =

$$\frac{initial\ weight - final\ weight}{initial\ weight} x\ 100\%$$

Moisture content (dry base) (%) =

$$\frac{initial\ weight - final\ weight}{final\ weight} x\ 100\%$$

Ash Content (AOAC, 2006)

Prepare the porcelain dish to do the obfuscation, then dry it in an oven for 15 minutes and then cool it in a desiccator and weighed (A). The sample is weighed ± 3 grams in a porcelain dish (B), then it is burned in chamber until it doesn't smoke again. Then put it in electric furnace at a temperature of 400 – 600°C for 4 – 6 hours until white ash was formed or had a fixed weight. The ash and cup are cooled in a desiccator then weighed (C).

$$\text{Ash Content (\% bb)} = \frac{(C - A)}{B} \times 100\ \%$$

Protein Content (AOAC, 2006)

Samples of ± 100 mg were weighed (A) and put into a 30 ml kjeldahl flask. Then added 1.9 ± 0.1 gr K_2SO_4, 40 ± 10 mg HgO, and 3.8 ± 0.1 ml H_2SO_4. Boiling stone is added to the flask and the sample is boiled for 1-1.5 hours until the liquid becomes clear. The contents of the flask and the rinsed water are transferred into a distillation instrument. The 125 ml erlenmeyer flask was filled with 5 ml of H_3BO_4 solution and added with 4 drops of indicators, then placed under a condenser with the tip of the condenser submerged in either H_3BO_4 solution. 8-10 ml NaOH-$Na_2S_2O_3$ solution was added to the distillation apparatus until it obtained distillate ± 15 ml in erlenmeyer. The distillate in the erlenmeyer is then titrated with 0.02 N HCl solution until there is a green to blue color change. Calculated the amount of nitrogen after previously obtained the amount of volume (ml) blank.

Amount of N (%) =

$$\frac{(\text{ml HCl} - \text{ml blank}) \times \text{N HCl} \times 14.007}{A} \times 100\ \%$$

Fat Content (AOAC, 2006)

Fat flask is provided according to the size of the soxhlet extraction tool used. The flask was dried in an oven at a temperature of 105 - 110 oC for 15 minutes, then cooled in a desiccator then weighed (A). Weighed as much as ± 5 g of sample (B) in filter paper, then covered with fat-free cotton. Filter paper and its contents are put into the Soxhlet extraction and installed on the condenser. The hexane solvent is poured into the Soxhlet flask to taste. Then it is refluxed for 5 hours until the solvent that comes back becomes clear. The solvent remaining in the fat flask is distilled and then the flask is heated in an oven at 105oC. After being dried to a fixed weight and cooled in a desiccator, the flask and the fat are weighed (C) and the following fat content is calculated.

$$\text{Fat content (\%)} = \frac{C - A}{B} \times 100\ \%$$

Carbohydrate Content by difference (AOAC, 2006)

Carbohydrate content is calculated based on by difference method with the following calculation:

Carbohydrate content (% ww) = 100% - (moisture content + ash content + protein content + fat content)

Microbiological Test

Escherichia coli Identification

Microbiological testing on fruits was carried out by quantitative identification of E. coli-Coliform. Where fruit samples are weighed 1 gram and dissolved in media (Modified Letheen Broth) 9ml MLB in aseptic conditions. Then 1 ml was taken to be planted on selective media for E. Coli-Coliform that is Cromocult Agar (CA). After it being solid, incubated at 36°C for 2x24 hours. Positive *E. coli* samples will grow purple colonies, and Coliform positive samples will grow red colonies.

Salmonella thypii Identification

Microbiological testing of fruits for the identification of *S. thypii* was carried out qualitatively. Fruit samples were weighed 25 grams and dissolved in media (Modified Letheen Broth) 225 ml in aseptic conditions. Then incubated at 36°C for 24 hours, to enrich microbes. Then 1 ml was taken to be planted on selective media for *S. thypii* that is Xylose Lysine Deoxycholate (XLD). After it being solid, it was incubated at 36°C for 2x24 hours. Positive samples of *S. thypii* will change the color of the media from brown to red and grow black colonies.

Statistical Analysis

The analysis technique used in this study is variance analysis (ANOVA), using the Statistical Package for Social Science (SPSS) application program. The design of the study used is Randomized Block Design, if it had a significant effect, it was further tested using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Fruits Screening

Roadside fruits may become contaminated with pathogenic microorganisms either during their growing in fields or greenhouses, or during harvesting, postharvest handling, and distribution (Beuchat, 2002).

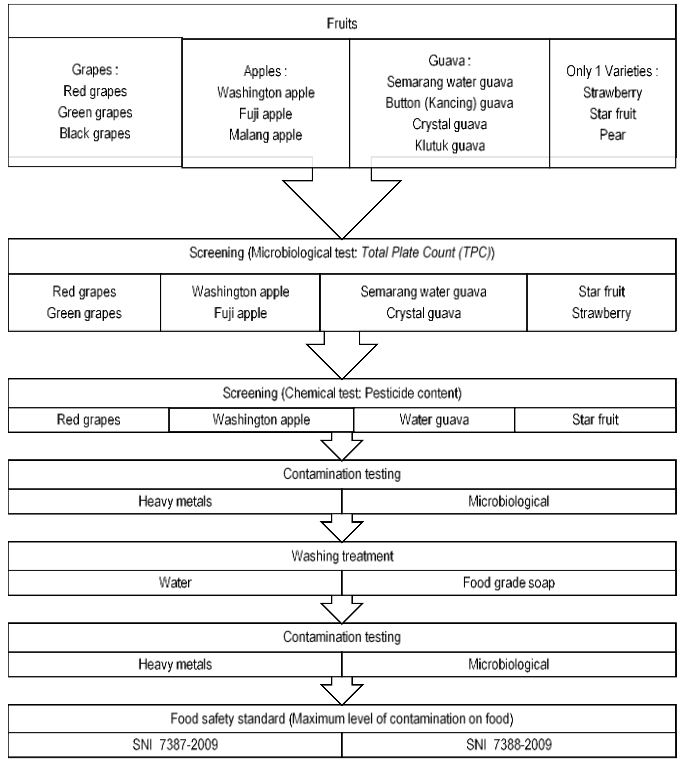

Figure 1. The steps of determining the effect of washing on heavy metal and microbiological contamination on fresh fruit

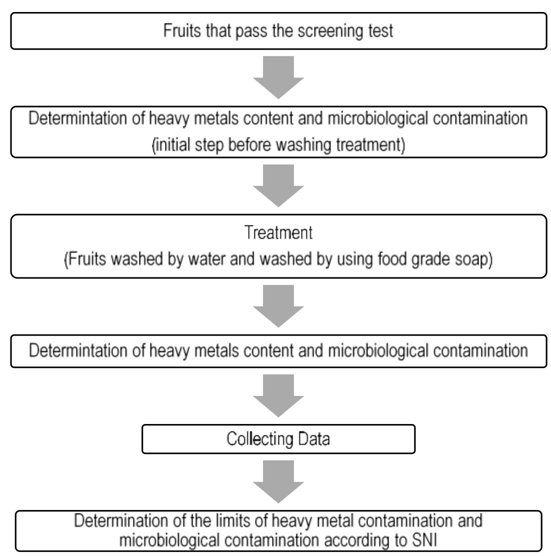

Figure 2. Flow Chart of determining the effect of washing on heavy metal and microbiological contamination on fresh fruit process

Postharvest sources of contamination include faeces, harvesting equipment, human handling, insects, wild and domestic animals, methods of transportation, processing equipment dust, and rinse water (Gil, et al., 2015). The result from fruits screening in microbilogical testing and chemical testing can be seen on Table 1 and Table 2. Table 1 and 2 showed that all roadside fruits showed bacterial, fungi, and yeast contamination, the most contaminated was grapes (red grapes and green grapes). Fruits like grapes, apple, guava, etc are mostly handled with bare hands during harvesting, packaging, and distribution; hence, it has been established that many fruits in the country are contaminated before they are sold, which, when not properly washed, will result in food-borne illnesses (Boateng, 2016). Each of the fruit varieties with the highest results of chemical analysis is selected one type that will proceed to the main study to determine the presence of heavy metal and microbiological contamination as well as the effect of washing on the decrease in content of heavy metal and microbiological contamination in fruits.

Table 1. Result from fruits screening in microbiological testing

No	Varieties	Sample	***Total Plate Count (TPC) colony/gram***		
			Bacteria	**Fungi**	**Yeast**
1	Grapes Vitis vinera	Red grapes	9.8×10^4	4.2×10^3	1.0×10^4
2		Green grapes	7.4×10^3	$<1 \times 10^1$	5.0×10^3
3		Black grapes	1.5×10^3	$<1 \times 10^1$	1.1×10^2
4	Apple Malus domestica	Washington apple	3.0×10^4	4.8×10^3	5.8×10^3
5		Fuji apple	1.6×10^4	1.8×10^3	1.3×10^3
6		Malang apple	4.7×10^3	3.0×10^2	2.5×10^3
7	Water guava Syzygium aqueum	Semarang water guava	2.2×10^5	1.3×10^3	2.5×10^3
8		Button guava (Jambu kancing)	1.1×10^5	$<1 \times 10^1$	$<1 \times 10^1$
9	Guava Psidium guajava	Crystal guava	1.8×10^5	2.3×10^3	1.2×10^3
10		Klutuk guava	1.4×10^4	2.0×10^3	1.2×10^3
11	Only 1 variety	Strawberry	2.4×10^4	5.3×10^3	2.5×10^3
12		Dewi star fruit	2.9×10^4	1.1×10^4	5.7×10^3
13		Pear	1.4×10^4	2.0×10^3	1.2×10^3

Table 2. Result from fruits screening in chemical testing

No	**Varieties**	**Sample**	**Result (mg/kg)**
1	Grapes	**Red grapes**	**0.90**
2		Green grapes	0.43
3	Apple	**Washington Apple**	**0.33**
4		Fuji apple	0.16
5	Guava	**Semarang water guava**	**0,07**
6		Crystal guava	0,03
7	Only 1 variant	Strawberry	0,03
8		**Dewi star fruit**	**0,06**

Heavy Metals Content

The results of the content of heavy metals analysis for Pb, Cd, and As from each fruit can be seen in the Table 3. Test results of heavy metal content in fruits can be seen from Table 3 have decreased. Test results of variance Pb heavy metal content in fruits showed that washing treatment (treatment significance = 0.204) did not significantly affect Pb content at the level of significance (α) 0.05. PB is very dangerous for the environment. The period of stay of Pb particles in the air is 4-40 days. Pb particles can be spread by the wind to reach distances of 100-1000 km from the source (Ayu, 2000). While Cd heavy metal, the test results of variance Cd heavy metal content showed that washing treatment (treatment significance = 0.371) did not significantly affect the levels of Cd heavy metals at the level of significance (α) 0.05. Cd is a toxic pollutant in water from metal mining, melting, plating, batteries, pesticides, oil paint, pigments, and alloys (Li et al., 2007 and Chen et al., 2008). Large dosages of Cd in humans can cause proteinurea and anemia (Horsfall et al., 2005).

Arsenic (As) is one of the major global environmental pollutants because of its highly toxic and carcinogenic properties (Bhattacharya, et al., 2010). The epidemiological studies show that the chronic As poisoning can cause cancers, diabetes mellitus (DM), hypertension, melanosis, hyperkeratosis, restrictive lung disease, peripheral vascular, and ischemic heart disease (Mazumder et al. 2000; Morales et al. 2000; Srivastava et al. 2001; Rahman 2002). While the test results of heavy metal content of As in fruits showed that washing treatment (significance of treatment = 0.020) significantly affected the content of As heavy metal at the level of significance (α) 0.05. Further testing in the form of Duncan test was carried out to see the effect of washing treatment on As content in the fruit group. The results of the test showed that the decrease in As content in fruits can be seen in Table 3. As the levels at the beginning before washing had a significant difference to the As content after washing using water only and food grade soap. The level of As in the fruit group after washing using water only and food grade soap has no real difference. This is because As has a difficultity to dissolve in water.

Table 3. Result of heavy metals analysis (mg/kg) on fruits

Heavy metals	Washing treatment	Grapes	Apple	Guava	Star fruit
Pb	Initiate	3,973[a]	0,216[a]	0,066[a]	1,027[a]
	Water treatment	1,390[a]	0,053[a]	0,000[a]	0,403[a]
	Food grade soap treatment	0,000[a]	0,000[a]	0,000[a]	0,023[a]
Cd	Initiate	1,177[a]	0,053[a]	0,000[a]	0,037[a]
	Water treatment	0,006[a]	0,000[a]	0,000[a]	0,034[a]
	Food grade soap treatment	0,000[a]	0,000[a]	0,000[a]	0,000[a]
As	Initiate	0,141[a]	0,141[a]	0,243[a]	0,074[a]
	Water treatment	0,089[b]	0,080[b]	0,103[b]	0,064[b]
	Food grade soap treatment	0,065[b]	0,041[b]	0,085[b]	0,053[b]

Microbiological qualities

The results of microbiological analysis of the identification of *E. Coli*-Coliform and *Salmonella thypii* can be seen in Table 4. The decrease in the number of colonies of *E. coli*-coliform and *S. thypii* due to washing treatment on fruits can be seen in Table 4. From three washing treatments used, it can be seen that washing treatment using food grade soap is better than washing using only with water to decrease the amount of E. coli-coliform and S. thypii colonies in the fruit group used. The results of the variance in the identification of *E. Coli*-Coliform in fruits showed that washing treatment (treatment significance = 0.127) did not significantly influence the decrease in the number of *E. Coli*-Coliform colonies at the level of significance (α) 0.05. While the results of the identification test for fruit *S. Thypii* showed washing treatment (significance = 0.000) significantly affected the decrease in the number of S. thypii at the level of significance (α) 0.05. Further testing in the form of Duncan test was conducted to see the effect of washing treatment on the number of *S. thypii* in the fruit group. The results of duncan test on the decrease of *S. thypii* in fruits can be seen in Table 4. It can be seen from the Duncan test results that the number of *S. thypii* at the beginning before washing and after washing using running water has no real difference, but both have real differences on the number of *S. thypii* after washing using food grade soap. This can be caused by the small number of *S. thypii* in fruits, so that antibacterial substances in food grade soaps are very effective for eliminating *S. thypii*.

Table 4. Result of microbiological identification (col/g) on fruits

Microbes	Washing Treatment	Grapes	Apple	Guava	Star fruit
E. coli	Initiate	$1x10^2$	$3,2x10^2$	$5,4x10^4$	$4,4x10^4$
	Water treatment	$3x10^1$	1,000	$1,8x10^4$	$6,0x10^3$
	Food grade soap treatment	0,000	0,000	$8,8x10^2$	$3x10^2$
S. thypii	Initiate	1^a (+)	1^a (+)	1^a (+)	1^a (+)
	Water treatment	1^a (+)	1^a (+)	1^a (+)	1^a (+)
	Food grade soap treatment	0^b (-)	0^b (-)	0^b (-)	0^b (-)

Supporting Test

Supporting tests on fruits were carried out to determine the nutritional quality of fruits and to determine the content of active ingredients and antibacterial substances in food grade soaps that have been tested. Proximate testing results and mineral content testing in fruits can be seen in Table 5. The results of the active ingredients content in food grade soap can be seen in Table 6. And the results of antibacterial substances testing on food grade soap can be seen in Table 7.

CONCLUSION

Chemical tests results showed that there was an effect (<α = 0.05) of washing treatment on heavy metal contamination in fresh fruits which are usually consumed directly without peeling the skin, and has a significant effect on Arsenic (As) heavy metal content. For the results of microbiological tests showed that there was an effect (<α = 0.05) of washing treatment on microbes in fresh fruit which is usually consumed directly without peeling the skin. And significantly affect the pathogenic microbes of Salmonella thypii. Based on the results of this study on fruits washing treatment using food grade soap which is usually consumed without peeling the skin and sold along the road is more effective in reducing heavy metal and microbiological contamination.

Table 5. Result test of proximate and mineral content in fruits

Parameter	Result			
	Grapes	**Apple**	**Guava**	**Star fruit**
Water content (%)	83.99	83,39	93,77	92,99
Ash content (%)	0,17	0,01	0,02	0,02
Fat content (%)	0.09	0.07	0.06	0,07
Protein content (%)	1,82	1,66	0,89	1,23
Carbohydrate content (%)	13,15	14,39	5,98	5,49
Fe (mg/100g)	0,326	0,132	0,073	0,077
Ca (mg/100g)	10,895	6,774	29,253	3,346
Mg (mg/100g)	7,141	5,116	5,586	10,583

Table 6. Result test of active agent (Anionic surfactant) in food

Parameter	**Result (%)**
Lauryl Alkyl Sulphonate Sodium *(LAS Na)*	10,85
Sodium Lauryl Ethoxy Sulphonate *(SLES)*	11,17

Table 7. Result test of active agent (Anionic surfactant) in food

Parameter	**Result (%)**
Triclosan	0
TCC	0
Methylisothializone	0
Methylchloroisothializone	0
Zinc Sulphate	1,06

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