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# Removal Efficiency of Microorganism and Pesticide Residues by a Using Surface Washing System on *Yuja* (*Citrus junos* Sieb ex Tanaka)

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# 표면 세척 시스템에 의한 유자의 미생물 및 잔류농약의 제거효과

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## Abstract

This study was conducted to assess the effects of the removal of pesticide residues and microorganisms from *yuja* (*Citrus junos* Sieb ex Tanaka) using a surface-washing system, under the following washing conditions: 0.11, 0.42, and 0.73 m/s spray rotation speeds; 0.6, 0.9, and 1.2 MPa water pressure and 0.046, 0.092, and 0.138 m/s conveying speeds. Tap-water treatment was used as the control. The washing efficiency when using squid ink was highly correlated with the conveying speed and the spray rotation speed. In addition, the highest washing efficiency was achieved when the water pressure was 0.9 MPa. The microorganisms were reduced to 0.40 log CFU/g for the tap-water treatment, and all the treatments, except those at the conveying speed of 0.138 m/s and the spray rotation speed of 0.11 rpm (6.07 log CFU/g), produced higher removal efficiencies compared with the tap-water treatment. Reductions of 2.20 and 2.05 log CFU/g were achieved at the spray rotation speeds of 0.42 and 0.73, respectively. The largest reductions were observed when the conveying speed was 0.046 m/s. Higher pesticide residue removal efficiency values were obtained at slower conveying speeds and higher spray rotation speeds. Higher than 50% removal efficiency was achieved when the spray rotation speed was 0.046 m/s for spirodichlofen, deltamethrin, benomyl, thiophanate-methyl, and acequinocyl. Especially, the removal efficiency for benomyl and thiophanate-methyl was more than 90%. It can thus be concluded that the pesticide residues in *yuja* can be effectively reduced by washing the latter with a less-than-0.092-m/s conveying speed and a higher-than-0.42-m/s spray rotation speed.

Key words : *yuja*, pesticide residues, microorganism, washing efficiency, removal efficiency

## Introduction

The use of pesticides is essential to control pests in horticultural crops, which are important to maintain an adequate food supply for an increasing world population. They are known to increase agricultural production tremendously as these chemicals act on pests that destroy agricultural produce (1). The crop yields which were cultivated without pesticides was about 30~80%. Especially, the yields of apple and peach were only 3 and 0%, respectively (2). However, agricultural pesticides can have an adverse effect on the environment and can have long-term health implications in humans. The organophosphorus pesticides are potent cholinesterase inhibitors and produce symptoms such as the over-stimulation of the central nervous system, which include restlessness as well as depression of the respiratory or cardiovascular system (3).

In general, food is the main route of exposure to pesticides. Especially, because fruit and vegetables are mainly consumed raw or semi-processed, it is expected that they contain higher pesticide residue levels compared to other food groups, such as products that are bread-based, which are subjected to cereal processing (4). Nam *et al.* (5) reported that residual pesticides were detected in 70 samples (19.7%) and 15 samples (4.2%) at concentrations exceeding the maximum residue levels

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(MRLs) in 355 vegetables. Han *et al.* (6) also reported that the detection rate of pesticides was 70.8% and that this rate exceeded the MRLs (1.67%) in 120 fruits. Consumers are very much concerned about the health risks associated with the occurrence of detectable pesticide residues in their food supply. The results from a survey of consumer recognition showed that 87.6% of the consumers believed that pesticide residues were very dangerous (7).

*Yuja* (*Citrus junos* Sieb ex Tanaka) is a citrus fruit native to northeast Asia, including Korea, China, and Japan (8). Particularly in Korea, it is commonly used as a raw material for beverages, such as *yuja*-tea, which is made from marmalade-like syrup with sugar and sliced *yuja* (9-10). The high content of vitamin C and phenolic substances in *yuja* might be associated with significant health benefits (11). It is already well known that citrus fruits are rich in flavonoids, most of which exist in the form of flavanone glycosides (FGs) such as narirutin, naringin, hesperidin, and neohesperidin (12). These phytochemicals have antioxidant capacity and may protect cells against oxidative damage caused by free radical. These phytochemicals and anti-carcinogenic properties have been shown to be higher in the peel than in the flesh of the fruit (13). Several studies have examined methods to remove pesticide residues from fruits (14-17) including washing, peeling, ozone treatment, cooking, etc. (18-22). However, no studies have examined the effects of mechanical washing on the removal of pesticide residues from *yuja*.

This study examined the efficiency of removing pesticide residues and microbes using a surface washing system with the goal of developing a method to produce safe *yuja*. The removal efficiency of pesticide residues and microorganisms was analyzed under different processing conditions including varying the spray rotation speed, conveying speed and water pressure.

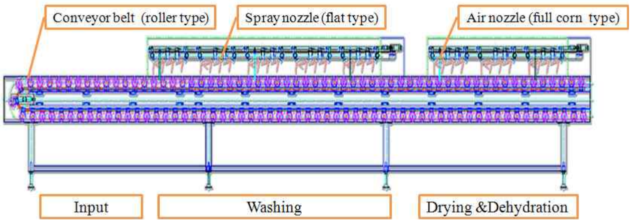
## Materials and Methods

### Materials

The *yuja* that was purchased and used in the experiment was harvested in Goheung, Jeolla, Republic of Korea in November 2009. *E. coli* (ATCC10536) was used for the microbe experiment. Seven types of pesticides were studied: chlorpyrifos, prothiofos, spirodichlofen, deltamethrin acequinocyl, benomyl and thiophanate-methyl. Each pesticide standard was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The pesticides were made in 3 L by diluting each pesticide in distilled water. It was then immersed into a dilute solution of pesticide for 10 minutes and dried for 24 hours and then used for pesticide analysis. The *E. coli* was cultured in a condition of 37 C. in a nutrient agar and broth (Difco, Flanklin Lakes, NJ, USA) for 24 hours. The *yuja* was immersed for 10 min in the culture solution and dried for two hours at room temperature and then used.

### Surface washing system

A surface washing system to clean the *yuja* was composed of an input, washing, dehydration and drying lines, as shown in Fig. 1. The conveyor belt for all lines consisted of a roller type, which was used to remove pollutants on the surface of round shape fruits such as *yuja*. The washing line consisted of a rotary spray with three flat-type nozzles and a piston vacuum pump to produce the washing pressure. The washing line was used to control the water pressure within 0.6-1.2 MPa at spray rotation speeds ranging from 0 to 0.73 m/s and conveyor speeds ranging from 0 to 0.138 m/s.



(a) Schematic diagram

(b) Picture

**Fig. 1. Surface washing system of *yuja*.**

### Experimental design

The first experiment was conducted to assess washing efficiency at water pressures of 0.6, 0.9, 1.2 MPa, spray rotation speeds of 0.11, 0.42, 0.73 m/s and conveyor belt speeds of 0.046, 0.092, 0.138 m/s. Tap water treatment was used as the control. All statistical analysis was performed using SAS (Statistical Analysis System, Cary, NC, USA). The RSM (response surface methodology) program was used to determine the optimum water pressure. The second set of experiments was performed to evaluate the removal efficiency of pesticide residues and microorganism. These experiments were conducted at a conveying speed of 0.046, 0.092, and 0.138 m/s, a spray rotation speed of 0.11, 0.42, 0.73 and water pressure of 0.9 MPa, which was selected based on washing efficiency experiment and described above (Table 1).

**Table 1. Experimental design for removal efficiency of pesticide residues and microorganism**

| Conveying speed (m/s) | Spray rotation speed (m/s) | Water pressure (MPa) |
|---|---|---|
| 0.046 | 0.11 | 0.9 |
| | 0.42 | |
| | 0.73 | |
| 0.092 | 0.11 | |
| | 0.42 | |
| | 0.73 | |
| 0.138 | 0.11 | |
| | 0.42 | |
| | 0.73 | |

### Washing rate of squid ink

Squid ink (*Ojingeo Meokmul*) was sprayed and analyzed the washing rate. The powder of squid ink from the market was diluted 10 times in distilled water and *yuja* was immersed in this solution for five minutes and then dried for one day at room temperature. The ink on the surface of the *yuja* was washed out with 50 mL of distilled water for the washing rate experiment and was measured at 450 nm using an UV-Vis recording spectrophotometer (V-530, Jasco, Tokyo, Japan). The absorbance was then measured and compared with the control.

$$\text{Washing rate (\%)} = \frac{\text{(Absorbance of initial group} - \text{Absorbance of washing group)}}{\text{Absorbance of initial group}} \times 100$$

### Microbial analysis

The *yuja* peel was placed into a sterilized bag and homogenized for one minute with 0.85% of sterilized saline solution. Each 1 mL of the diluted solution was put into petri dishes for the experiment, according to the pour-plate method. The chromacult agar (Merck, Darmstadt, Germany) was used as the culture medium and the plates were incubated at 37℃ for 24 hr. Colonization data were converted to log CFU per gram of fresh weight.

### Pesticide residue analysis and recovery study

The analysis of pesticide residue followed the method of the Official Analytical Method of Pesticides (23). 20 g of *yuja* peel was put into a 300 mL beaker and was extracted for 1 minute by an homogenizer with 100 mL acetonitrile. The extracted solution was then filtered and put in the separation bottle with 10 g of NaCl and was left for three hr for layered separation after shaking. 20 mL of the supernatant was taken and then evaporated under a stream inside the water bath under 40℃. The florisil cartridge (Waters, Milford, MA, USA) was preconditioned with 5 mL of hexane followed by 5 mL of 20% aceton/hexane. The extract of pesticides was put into a cartridge and 10 mL of 20% aceton/hexane were used to clean up the residue. After evaporating the extract again, it was dissolved with 2 mL of 20% aceton/hexane for analyzing GC (Gas chromatography). An $NH_2$ cartridge was preconditioned with DCM (dichloromethane) 5 mL, followed by DCM containing 1% methanol. Then the pesticide extract was evaporated and dissolved with 2 mL of acetonitrile for analyzing for HPLC (High performance liquid chromatography). The analysis condition of GC and HPLC is shown in Tables 2-4. The results of recovery test on untreated *yuja*, using the pesticide, were spiked with standard pesticide at 1.0 mg/kg. The recovery rates were 97.41~107.66% and judged to be satisfactory.

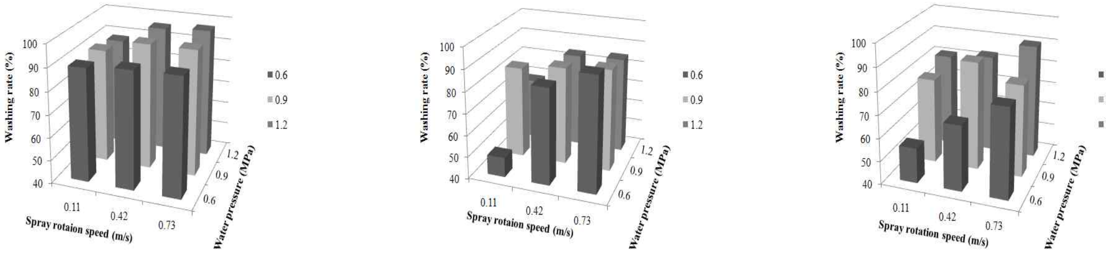
**Table 2. GC condition for the analysis of the pesticide residues**

| Instrument | GC 2010 (Shimadzu, Kyoto, Japan) |
|---|---|
| Detector | ECD |
| Column | DB-5 (30 m × 0.25 mm × 0.25 μm) |
| Injector temp. | 260℃ |
| Detector temp. | 280℃ |
| Oven temp. | 120℃ 2 min (10℃/min)<br>220℃ 2 min (7℃/min)<br>250℃ 2 min (7℃/min)<br>280℃ 15 min |
| Carrier gas | N2 |
| Column flow | 1.0 mL/min |
| Split ration | ECD:split mode 20:1 |
| Injection volume | 2 μL |

## Results and Discussion

### Washing rate of squid ink

The washing rate is shown in Fig. 2. The washing rate at a conveying speed of 0.046 m/s was 88.06~97.07%, which was higher than the washing rate at a convey speed of 0.092 m/s (49.14-92.92%) and 0.138 m/s (55.34-90.05%) regardless



(a) 2.76 m/min (b) 5.52 m/min (c) 8.28 m/min

**Fig. 2. Washing rate of *yuja* by various washing conditions.**

**Table 3. HPLC condition for the analysis of the acequinocyl**

| Instrument | UFLC (Shimadzu, Kyoto, Japan) |
|---|---|
| Column | Shiseido capcell pak C18 UG120<br>(4.6 mm×250 mm, 5 μm) |
| Detector | UV detector 250 nm |
| Column Temp. | 40℃ |
| Mobile phase | Aqueous 0.01% phosphoric acid : Acetonitrile = 120 : 880 |
| Column flow | 1.0 mL/min |
| Injection Volume | 20 μL |

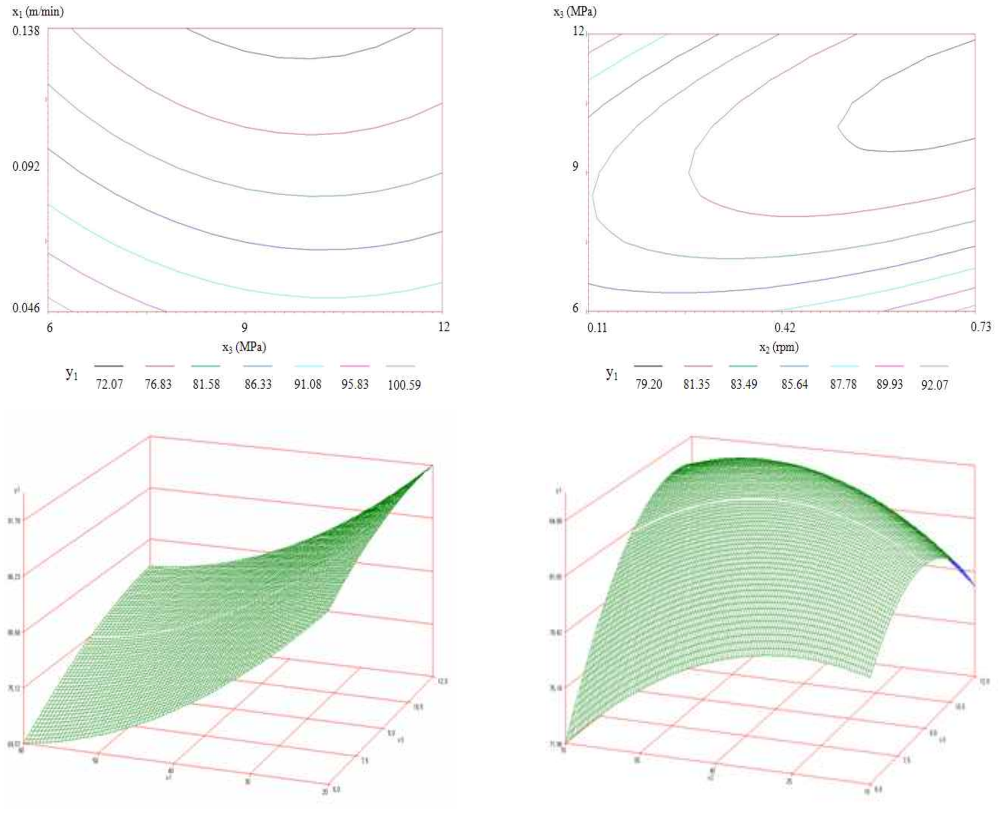
of the water pressure and spray rotation speed. The washing rate at a conveying speed of 2.76 m/min, water pressure of 1.2 MPa and spray rotation speed of 0.42 and 0.73 m/s were 95.88% and 97.07%, respectively, which was the highest among the washing conditions. The next highest washing rate was 95.24% and 95.25%, which was achieved at a water pressure of 0.9 MPa and spray speed of 0.42 and 0.73, respectively. In addition, no significant difference in removal efficiency was observed when the water pressure was 1.2 MPa. The washing efficiency ranged between 82.67-87.45% when the conveying speed was 0.092 m/s and water pressure was 0.9 MPa, which was higher than the efficiency obtained when the water pressure was 1.2 MPa (68.63-84.81); however, this difference was not significant ($p<0.05$). In the case of a conveyor speed of 2.76 m/min, the washing rate at a water pressure of 0.9 MPa (90.09-95.25%) was similar to the water efficiency obtained at a water pressure of 1.2 MPa (88.06-97.07). The correlation between the washing rate, conveying speed, spray rotation speed and water pressure is shown in Table 5. The washing rate was highly correlated with the conveying speed and spray rotation speed. According to these results, the washing rate increased when the conveying speed was lower and the spray rotation speed higher. However, the washing rate was not correlated with water pressure. The optimal condition of water pressure was at 0.9 MPa through the response surface analysis, because the highest washing efficiency of water pressure ranged from 0.9 to 1.2 MPa (Fig. 3).

**Table 4. HPLC condition for the analysis of the benomyl and thiophanate-methyl**

| Instrument | UFLC (Shimadzu, Kyoto, Japan) | | |
|---|---|---|---|
| Column | Shiseido capcell pak C18 UG120<br>(4.6 mm×250 mm, 5 μm) | | |
| Detector | UV detector 254 nm | | |
| Column Temp. | 40℃ | | |
| | Time (min) | Water (%) | Acetonitrile (%) |
| | initial | 95 | 5 |
| | 7 | 95 | 5 |
| Mobile phase | 15 | 80 | 20 |
| | 30 | 10 | 90 |
| | 40 | 10 | 90 |
| | 45 | 95 | 5 |
| Column flow | 1.0 mL/min | | |
| Injection Volume | 10 μL | | |

### Microorganisms

Experiments on the reduction of microorganisms were conducted based on the washing efficiency experiments. The conditions used for these studies were as follows: conveying speeds of 0.046, 0.092, 0.138 m/s spray rotation speeds of 0.11, 0.42, 0.73 and a water pressure of 0.9 MPa. The microbial reduction rate are shown in Fig. 3. The initial microbe concentration was in 6.40 log CFU/g. Microorganisms increased at higher conveying speeds and spray rotation speeds. In addition, except at a conveyor speed of 0.138m/s and spray rotation speed of 0.11 m/s (6.07 log CFU/g), microbial removal was higher for the treated groups than tap water washing group. The largest reductions (2.20 and 2.05 log CFU/g) were observed at a rotation spray speed of 0.42 and 0.73 m/s, respectively, when the conveying speed



$x_1 \times x_3$ $x_2 \times x_3$

**Fig. 3. Response surface and contour map of conveying speed (x1)×water pressure (x3) and spray rotation speed (x2)×water pressure (x3) on washing rate (y1) for determining the optimum condition of water pressure.**

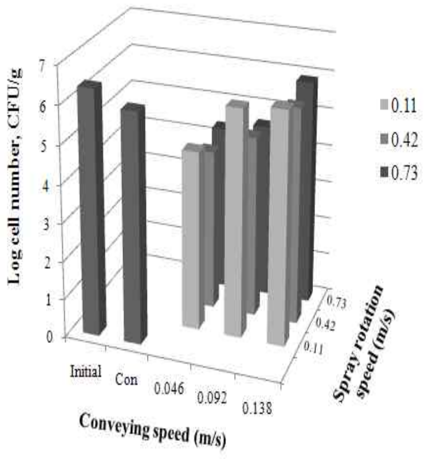


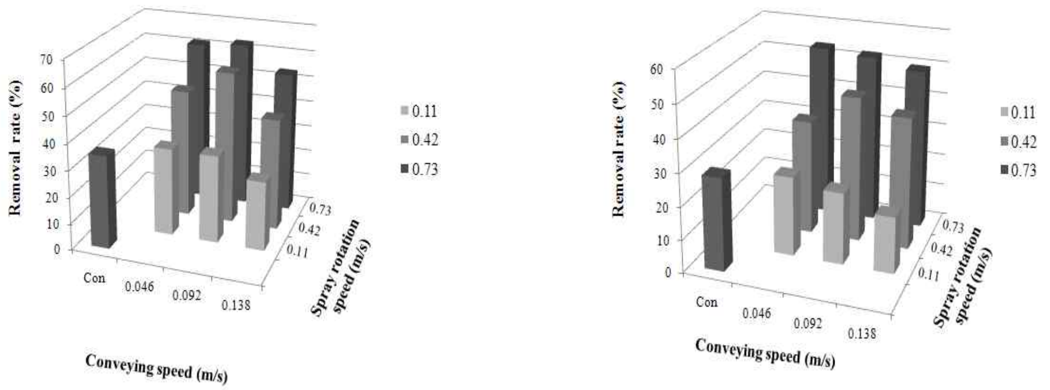
**Fig. 4. Effect of washing on microorganism in *yuja*.**

was 0.046 m/s. Lee *et al.* (24) reported that *E. coli* and *B. cereus* counts of vegetables decreased 1~2 log CFU/g with micro-bubbles washing. Also the total bacterial counts of peach decreased 1 log CFU/g with physical washing method (25). It was considered that the best conditions were above 0.42 m/s, a rotation spray speed and below 0.092 m/s, conveying speed for eliminating more than 2 log CFU/g of microorganisms.

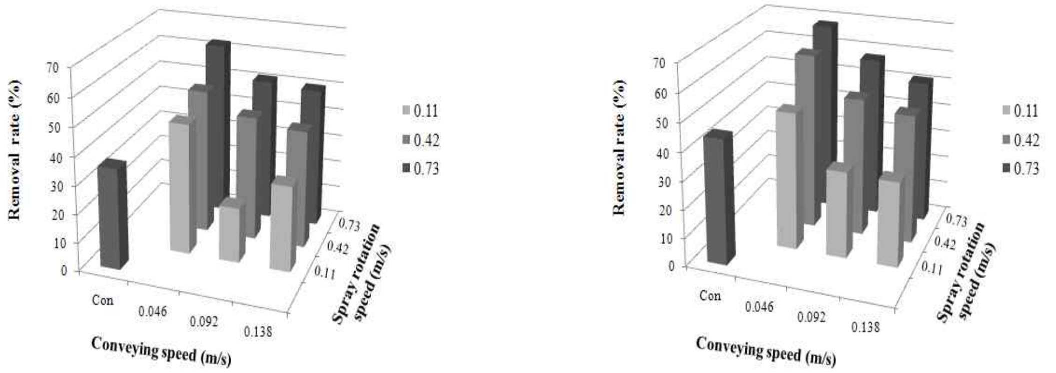
### Pesticide residues

The excessive use of pesticides results in widespread environmental problems such as a disturbance of natural balance, pesticide resistance, and hazard to a human being. It has been reported peeling could reduce the pesticide residues from vegetables and fruits significantly (26). But *yuja* need more effective washing methods for removing pesticides, because both flesh and peel of the fruit are used.

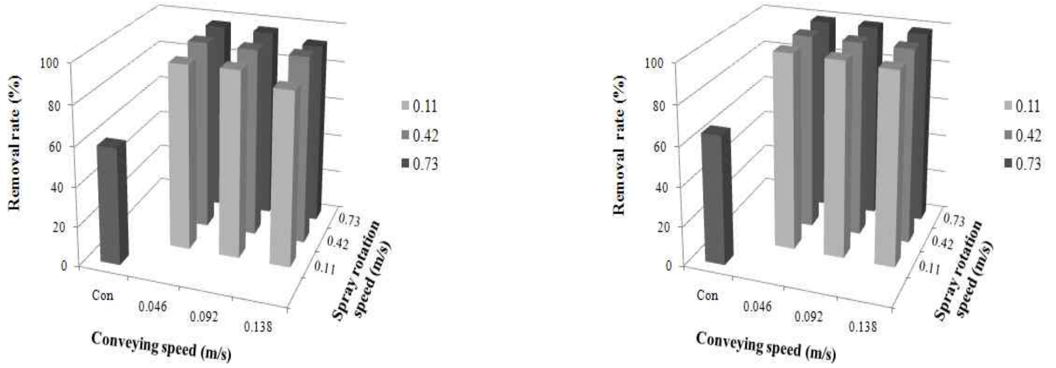
The surface washing system was used as an alternative to reduce pesticide residues and the result is shown in Fig. 5. The chlorpyrifos was reduced to 34.99% after tap water treatment. When the apples were cleaned with tap water, a similar reduction ratio (39.35%) as reported by Choi *et al.* was observed (27). A higher removal ratio was observed for the treated samples than tap water with a reduction of more than 42.19% when the spray rotation speed was 0.42 m/s, regardless of the conveying speed. The removal ratio was



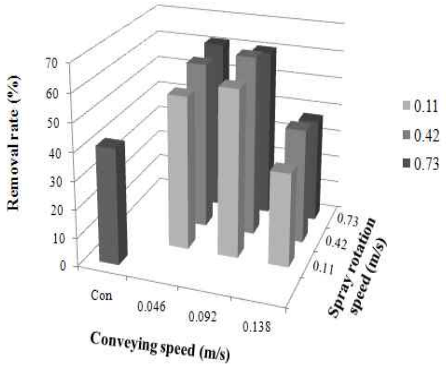
(a) Chlorpyrifos (b) Prothiofis



(c) Spirodiclofen (d) Deltamethrine



(e) Benomyl (f) Thiophanate-methyl



(g) Acequinocyl

Fig. 5. Removal rate of pesticide residues on *yuja* by various washing conditions.

62.87% when the conveying speed was 0.092 m/s and spray rotation speed was 0.73 m/s, which was higher than the ratio obtained at a spray rotation speed of 0.43 m/s (57.63%); however, this difference was not significant. The pesticide residue was reduced to 61.31% when the conveying speed was 0.046 m/s and the spray rotation speed was 0.73 m/s, which was not significantly difference from when the spray rotation speed was 0.92 m/s (62.87%) (Fig. 5-(a)). Yoon *et al.*(14) reported that the maximum removal rate of chlorpyrifos on apples using an ultrasonic cleaner was 39.2%. The removal efficiency of prothiofos ranged from 24.37-53.76% when the rotation speed was 0.42 and 0.73 m/s, which was higher than tap water treatment. For prothiofos, the removal rate were 53.76, 52.04 and 49.48% in 0.046, 0.092 and 0.138 m/min when the spray rotation speed was 70 rpm; however these values were not significantly different ($p<0.05$) (Fig. 5-(b)). The chlorpyrifos residue concentration in fresh, fruit skin and stalk cavity of apples for 15 days after harvesting were reported to be 0.1%, 22.8% and 77.1%, respectively (28). It is known that organophosphorus pesticides such as chlorpyrifos and prothiofos, which are dissolved in the wax layer, are not easily removed by washing. Organophophorus pesticide residues translocate into internal tissue and may not be removed physically and chemically. Penetration depends on the stability of the insecticidal material in the lipoid-like or waxy-like layer, which covers the cuticle. The thicker and oily the skin, the more likely it can penetrate (29, 30). Peeling can reduce the level of pesticide residues on fruits and vegetables; however, an effective washing method is needed of *yuja* since all parts of this fruit, including the peel and flesh, are eaten. The best condition to remove spirodichlofen was a conveying speed of 0.046 m/s of conveying speed and spray rotation speed of 0.73 m/s. Under these conditions, the removing ratio was 62.52% (Fig. 5-(c)). The removal rate of deltamethrin under the different conditions is shown in Fig. 5-(d). The surface washing system produced a higher removal ratio than tap water washing (44.36%), except when the spray rotation speed was 0.11 m/s. When the spray rotation speed was 0.42 and 0.73 m/s, and the conveying speed was 0.046 and 0.092 m/s, the removal rate ranged from 49.36 to 67.91% however, no significant differences were observed between these conditions ($p<0.05$). A reduction of more than 65% was observed after tap water washing for benomyl and thiophanate-methyl and significantly higher reduction (95.52-99.47%) were observed when the washing system was used under all conditions tested compared with tap water washing (Fig. 5-(e), (f)). The removal rate of acequinocyl is shown in Fig. 5-(g). It showed a significantly high reduction of 54.57-61.40% when the conveying speed was 0.092 m/min compared with the reduction levels achieved using tap water washing (41.10%). The removal rate at conveying speeds of 0.046 and 0.092 m/s and spray rotation speeds of 0.42, 0.73 m/s ranged between 59.72 and 61.40% and a significant difference among the washing conditions was not observed ($p<0.05$). Thus, using this washing system, the pesticide residues in *yuja* can be effectively reduced when it cleaned at a conveying speed lower than 0.092 m/s and spray rotation speed greater than 0.42 m/s. The removing ratios of benomyl and thiophanate-methyl (above 90%) were higher than those of other pesticides. Benomyl and thiophanate-methyl are benzimidaxole family and highly permeable pesticides. Kwon *et al.* (14) previously reported that the thiophanate-methyl removing ratio was highest in cherry tomatoes. The permeability of the agricultural product, rather than the water solubility of the pesticide itself, influenced the removing ratio of pesticide residues by cleaning. The chlorpyrifos (water solubility, 1.4 mg/L) and prothiofos (water solubility, 0.7 mg/L) have a higher solubility compared with the other pesticides used in this study, but the removing ratio was not high. Krol (31) previously reported that there was no correlation between cleaning power and solubility. The removal rates of benzimidazole pesticides were 1.5~2.0 times bigger than that of organophosphorus pesticides. Jung *et al.* (32) reported that the chemical washing solution was effective to destroy pesticides by oxidation. Especially it easily removed pesticides with s=p and p=o double bonds such as chlorpyrifos, prothiofos, spirodichlofen, deltamethrin. Therefore, it is considered that the combined washing with chemical washing solution and washing system needs further research.

**Table 5. Correlation coefficients between washing efficiency and washing conditions**

| | Conveying speed | Spray rotation speed | Water pressure |
|---|---|---|---|
| Washing efficiency | $-0.5495^{***}$ | $0.4851^{***}$ | 0.1392 |

$^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$.

## 요 약

본 연구는 표면 세척 시스템을 이용하여 유자의 미생물과 잔류농약 제거 효과를 살펴보았다. 선행 연구로 오징어 먹물을 제거 효과를 보기 위해 스프레이 회전 속도 0.11, 0.42, 0.73 m/s, 수압 0.6, 0.9, 1.2 MPa과 컨베이어 속도

0.046, 0.092, 0.138 m/s 으로 세척 조건을 달리하였다. 오징어 먹물 제거 실험 결과 먹물 제거 효율은 컨베이어 속도와 분사 노즐 회전수와는 높은 상관관계를 나타내었으며 수압의 조건에서는 반응표면 분석 결과 0.9 MPa에서 가장 제거율이 높게 분석되었다. 이에 미생물과 잔류농약 제거률 실험의 위해 세척 조건으로 수압을 0.9 MPa로 고정하였으며 스프레이 회전 속도(0.11, 0.42, 0.73 m/s)와 컨베이어 속도(0.046, 0.092, 0.138 m/s)를 달리하여 수행하였다. 미생물은 컨베이어 속도 2.76 m/min 일 때 스프레이 회전속도 0.43과 0.73 m/s에서 각각 2.20, 2.05 log CFU/g 수준 감소하여 가장 높은 감소율을 나타내었다. 또한 스프레이 회전속도 0.11 m/s와 컨베이어 속도 0.138 m/min을 제외한 모든 처리구의 미생물 제거효과는 수도수 처리보다 높았다. 잔류농약 실험 결과 컨베이어 속도가 느릴수록, 분사노즐 회전수가 많을수록 제거효과가 높았으나 spirodichlofen, deltamethrin, benomyl, thiophanate-methyl과 acequinocyl 의 경우 컨베이어 속도 0.046 m/s에서 분사노즐 0.42 m/s이상에서 처리구들 간에 유의적인 차이를 보이지 않았다. Benomyl과 thiophanate-methyl 의 잔류 농약은 컨베이어 속도 0.092 m/s 이하의 조건에서 90% 이상 제거되었다. 이상의 결과에서 표면 세척 시스템의 조건을 컨베이어 속도 0.092 m/min 이하, 노즐 회전수 0.043 m/s 이상에서 세척 시 유자의 미생물과 잔류농약을 효율적으로 제거할 수 있을 것으로 판단된다.

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