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# Removal of pesticide residue from Brussels sprouts by hydrostatic pressure

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ABSTRACT

Food safety concerning polluted products has been of considerable interest in recent years. In our previous study, a pressurization technique was applied to cherry tomatoes laced with pesticides, and found that hydrostatic pressure treatment (HPT) reduces the amount of pesticides in samples. The objective of this study is to investigate whether HPT will affect Brussels sprouts, which have a rougher surface than cherry tomatoes. Samples were treated at several pressures (0.1–400 MPa) and at two temperatures (5 or 25 °C) for 30 min. Pesticide removed from samples accumulated in the surrounding water. Moreover, HPT was performed with ethanol solution as the surrounding medium, resulting in complete pesticide removal at comparatively low pressure. Under these conditions, visual changes did not occur, toxic intermediates from the pesticide were not detected, and nutrients from the samples were not found in the surrounding medium.

*Industrial relevance:* This article demonstrates that HPT with 10% ethanol solution is a potentially safe and harmless washing technology. It can remove pollutants from Brussels sprouts, which can then be collected from the surrounding medium, without breaking down the pollutant into more toxic materials. We believe that this washing process will be of interest to those involved with food safety, and may eventually be used for food safety during food production.



## 1. Introduction

The safety and quality of food products are among the most important factors influencing consumer choices in modern times; they are also the most important considerations of food manufacturers and distributors (Cardello, Schutz, & Lesber, 2007; Ohlsson, 1994). Problems associated with food safety include illnesses, deaths, product recalls, industry bankruptcies, job losses, overall economic losses, and tension in international relations. It is therefore of utmost importance for the food industry to continue to seek out more effective methods to remove pollutants from products and reduce undesirable changes in foods associated with food processing. The pollutants may include chemicals that cause acute or long-term toxicity, biological agents such as pathogenic bacteria, viruses, parasites and abnormal prions causing transmissible spongiform encephalopathies, or physical objects. It is well known that soluble pollutants on the surface of products are easily washed off with water, while insoluble pollutants persist on food and potentially threaten human health (Chavarri, Herrera, & Arino, 2005; Mukherjee, Kole, Bhattacharyya, & Banerjee, 2006; Nagayama, 1996; Zhang, Liu, & Hong, 2007). In addition, pollutants infiltrated into food products also persist after washing.

There have been several reports on methods for washing food products in which pesticides were present as pollutants. Pesticides can be roughly classified as hydrophilic (water soluble) or hydrophobic (water insoluble); the latter exhibits higher residual levels in food production. Several washing solutions such as chlorine solution, ozonated water and strong acid have been proven to successfully remove even hydrophobic pesticide residues during the commercial crop process (Ikeura, Kobayashi, & Tamaki, 2011a, 2011b; Ong, Cash, Zabik, Siddig, & Jones, 1996; Pugliese et al., 2004; Wu, Luan, Lan, Lo, & Chan, 2007; Zohair, 2001). Other reports have concluded that the water solubility of pesticides does not play a significant role in their removability in different commodities by washing (Cabras et al., 1997; Guardia-Rubio, Ayora-Cañada, & Ruiz-Medina, 2007; Krol, Arsenault, Pylypiw, & Incorvia Mattina, 2000), and partition coefficients between cuticle and water were found to correlate well with octanol/water partition coefficients, as reported by Baur, Marzouk, Achönherr and Grayson (1997). While the residue removal mechanism is complicated, the issue seems to be generally based on the form, material structures and chemical composition of the individual residue; the main factor is likely intermolecular interactions, so that weakening the hydrophobic bonds should be thought as important.

Hydrostatic pressure treatment (HPT) is a pressurization process conducted in a vessel filled with medium at pressures in excess of 100 MPa, with equal forces in all directions. This process is effective for inactivating most vegetative pathogens and spoilage bacteria that are commonly found in foods (Yuste, Capellas, Reyes, Fung, & Mor-Mur, 2001). The same pressurization process is one of the emerging technologies being investigated to enhance the safety and shelf life of many

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perishable foods (Ananth, Dickson, Olson, & Murano, 1998; Büyükcan, Bozoglu, & Alpas, 2009; Garriga, Grebol, Aymerich, Monfort, & Hugas, 2004; Knorr, 2002). Furthermore, this treatment is expected to be less detrimental than thermal processes to compounds in low molecular weight food, such as flavoring agents, pigments, and vitamins, as covalent bonds are not affected by pressure (Hayashi, 1992; Tauscher, 1995). Free water molecules at high pressure are stabilized by combining with ions, non-polar groups, and polar groups (Hayashi, 1991). Consequently, hydrophobic bonds and interactions are weakened at high pressure. As for the removal effect, hydrostatic pressure technology has been reported to extract internal substances, such as allergens (Kato, Katayama, Matsubara, Omi, & Matsuda, 2000; Kinefuchi, Yamazaki, & Yamamoto, 1995; Yamaguchi et al., 1995; Yamazaki & Sasagawa, 1997).

A pressurization technique was applied to cherry tomatoes covered with pesticides, and HPT helped to reduce pesticide levels in the tested samples. The pesticides accumulated in the surrounding of the samples after HPT. In a previous study, the optimum pressurization conditions of approximately 75 MPa and 5 °C resulted in a removal rate of nearly 75% from cherry tomatoes laced with pesticides (Iizuka, Maeda, & Shimizu, 2013). Iizuka, Yahata and Shimizu (2013) proposed a possible mechanism for this whereby it takes a long time to dissolve pesticides in water under high pressure. The same applies for utilization in food products laced with both high and low pesticides to the extent. Additionally, it was reported that HPT with 10% ethanol solution resulted in complete removal without breaking down the pesticides into more toxic materials, the elution of nutrients from the cherry tomatoes, or visual changes (Iizuka & Shimizu, in press). However, few reports have discussed the application of hydrostatic pressure as a method for washing food products, and the experiment on cherry tomatoes was only performed. Thus, in the present study, Brussels sprouts were selected, which are leafy vegetables with a rough surface, as the target food of washing. The objective of the present study was to examine whether the complete removal of pollutants from Brussels sprouts is possible, and whether HPT can remove the pollutants without damaging the sprouts. Chlorpyrifos (CP) was employed as the pollutant, which is a hydrophobic pesticide widely used in farming and detected in various foodstuffs, for comparison with our previous work.

## 2. Experimental

### 2.1. Samples

Brussels sprouts (species: *Brassica oleracea*) were purchased from a supermarket in Hachioji city, Japan. According to GC/MS (Gas Chromatography Mass Spectrometry) analysis, the samples used for all assays did not contain any pesticide residue. After purchase, the sprouts were maintained at approximately 4 °C until use (maximum of 1 day).

### 2.2. Chemicals

The pesticide-standard CP, with purity up to 98%, was purchased from Sigma-Aldrich (Steinheim, Germany). Dursban™ 40 EC containing 40% (w/v) CP for preparing the pesticide coating for the vegetable samples was obtained from Dow Agrosciences (Indianapolis, USA). The physical–chemical properties of CP are as follows: water solubility (25 °C) is 1.4 mg/L, water half-life (pH 7, 25 °C) is 72 days, and log *P*, which is the octanol–water partition coefficient, is 4.7. Methidathion (DMTP), which was used as an internal standard for GC/MS, was from Supelco Ltd. (Bellefonte, USA). Analytical grade acetone, dichloromethane, hexane and ethanol were from Wako Pure Chemical Industries (Osaka, Japan).

### 2.3. Treatment with pesticide

First, CP was sprayed on the Brussels sprouts; however, as reported elsewhere, the amount of pesticide residue varies widely with this approach (Yamashita, Noma, & Honda, 2009). Thus, a method of immersing the sprouts in the pesticide solution was adopted. This treatment was in accordance with the model of Pugliese et al. (2004). Our intention was to produce levels of residue approximating the maximum residue limit (MRL) set in the European Union, Unites States, China and Japan; the MRL for CP in Brussels sprouts is 1.0 mg/kg. Dursban™ 40 EC was diluted in tap water and used to spike samples with CP. Five samples were then selected and steeped for 1 min in this solution. Subsequently, the samples were left for 24 h at room temperature in a fume hood. The control sample did not undergo HPT after being coated with the pesticide.

### 2.4. Hydrostatic pressure treatment

Hydrostatic pressure treatment (HPT) was performed in a custom laboratory-scale vessel (capacity: 295 $cm^3$; maximum pressure: 500 MPa). Kerosene was used as the pressure-transmitting medium. The temperature of this high-pressure vessel was controlled by circulating water at a constant temperature. The rate of pressure increase was approximately 100 MPa per minute, and the releasing time was just a few seconds.

Each sample was packed in a polyethylene terephthalate pouch (60 × 85 mm). Each pouch was filled with 20 mL of water, 10% ethanol solution or 70% ethanol solution, and then sealed. These pouches were set in the high-pressure vessel filled with water as the pressurizing medium. The samples in pouches were pressurized at 25, 50, 75, 100, 200, 300 and 400 MPa at 5 °C or 25 °C for 30 min. Immediately after HPT, samples were rinsed for 15 s with tap water, while the surrounding water of the samples was collected for recovery of and analysis for CP. All analyses were performed in quintuplicate (Table 1).

### 2.5. Extraction processing

Five whole samples were homogenized using a commercial food processer (capacity: 500 mL; rotational speed: 800–3000 rpm), which is a kitchen appliance used to facilitate repetitive tasks such as cutting and mixing in the preparation of food. A 20-g portion of slurry sample was weighed in a 200-mL beaker and extracted in 100 mL of acetone for 30 min. The extract was filtered with a glass filter (GF/A; 55 mm) under reduced pressure and clean up was performed using a Chem Elut diatomaceous column. The glass filter and column were washed twice with 10 mL of acetone. The elution was concentrated to dryness in a vacuum rotary evaporator with a water bath at 60 °C, then reconstituted to 10 mL with a mixture of dichloromethane:hexane (1:4, v/v) for instrumental analysis; 500 μL of 200 ppm DMTP was added as an internal standard.

**Table 1**
Residual amount of Chlorpyrifos (CP) per kilogram of Brussels sprouts after hydrostatic pressure treatment. These results show the removal effect of pesticide for different pressurization conditions (0.1–400 MPa, 5 and 25 °C for 30 min) with water. All values are the mean ± standard error (n = 5). Different letters imply significant changes ($P < 0.05$).

| CP amount of non treatment (mg/kg) | High hydrostatic pressure | | |
|---|---|---|---|
| | Pressure (MPa) | 5 °C | 25 °C |
| | | CP residues (mg/kg) | CP residues (mg/kg) |
| 1.06 ± 0.04 | 0.1 | 0.64 ± 0.01 a* A** | 0.64 ± 0.06 a A |
| | 25 | 0.22 ± 0.02 b A | 0.25 ± 0.03 b A |
| | 50 | 0.21 ± 0.04 b A | 0.24 ± 0.01 b A |
| | 75 | 0.17 ± 0.03 bc A | 0.24 ± 0.05 b A |
| | 100 | 0.14 ± 0.01 c A | 0.27 ± 0.07 b B |
| | 200 | 0.12 ± 0.01 c A | 0.29 ± 0.04 b B |
| | 300 | 0.25 ± 0.03 b A | 0.31 ± 0.02 b A |
| | 400 | 0.53 ± 0.02 a A | 0.51 ± 0.04 a A |

* Same small letter on the same row indicated no significant difference between treatment groups.
** Same capital letter on the same tier indicated no significant difference between controlled temperatures (5 or 25 °C) on the treatments at the same pressurization.