

Research Article

Kinetics of the reduction of pesticide residues in vegetables by ultrasonic cleaning

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Abstract

The problem of pathogen and pesticide residues in vegetables is a big concern to both consumers and exporters. This study introduced a new method of cleaning vegetables with high frequency sound waves (ultrasonic) as a way to solve the problem. This research aimed at studying the kinetics of reduction of pesticide residues in four kinds of vegetables: coriander, kale, yard long bean and red chili by using the ultrasonic cleaner (60 kHz 140 W) with tap water compared with soaking in tap water. The cleaning was done at 20, 25 and 30°C for 0, 3, 7 and 10 minutes. The determination of the toxicity levels was done by means of cholinesterase inhibition technique. The decimal reduction time, the reaction rate constant and the activation energy were determined. It was found that ultrasonic cleaning at 25°C and soaking in tap water at 30°C was the best combination to reduce pesticide residues. The study concluded that ultrasonic cleaning was a far better method for reducing pesticide residues than soaking in tap water.

Keywords: high frequency sound waves, food contamination, toxicity, cholinesterase inhibition, cavitation, Thailand.

Introduction

Thailand is a large exporter of agricultural produce and processed agricultural products. However, these products are often rejected by trading partners because of the detection of microbial pathogens and pesticide residues, especially coriander, kale, yard long bean and red chili. The Department of Agriculture [1] reported that during January - July 2009, compared to the same period the previous year, exports of agricultural products and agro-industry products decreased, with about 29% of the EU-destined products found to have problems as reported by RASFF (Rapid Alert System for Food and Feed). Products found to have problems increased on the RASFF list from 40 to 74 items.

This problem occurred because vegetable production currently uses a lot of pesticides and cleaning is less effective. Good cleaning practice also helps to reduce dirt, pesticide residues and microbial contamination from raw materials. Ultrasonic cleaning is a new technology applied to wash vegetables and fruit in the food industry [2]. The frequency of ultrasound causes a reaction known as cavitation which can eliminate the residual pesticide residue, bacteria and dirt better than other methods, such as a spray cleaning, brush cleaning and water and air turbulence etc. [3]. The cavitation is a phenomenon of the fabrication of very small bubbles (not visible to the naked eye) in a liquid. These bubbles will grow and become filled with vapour, gas or a mixture thereof, expand and regularly break out violently under the stimulus of the ultrasound. These bubbles cause mechanical energy as shockwaves as well as particle mixing to remove dirt, pesticide residues and microorganisms [4, 5]. The effectiveness of the cleaning depends on the number of bubbles imploding and the implosion force of bubbles. The latter depends on amount of vapour and gas in each bubble. The less vapour and gas in the bubble, the cavitation intensity is increased and cleaning is more efficient [6]. Further, the cleaning efficiency depends on the temperature, ultrasound frequency, power level, the size, depth and shape of the cleaning bath, properties of the liquid and duration time of cleaning [4].

One detection method for pesticide residues is by measuring the degree of toxicity which be done by GT-test kit which is a semi-fast testing method using the cholinesterase inhibition technique [7]. The theory suggests that the toxicity in the organophosphate and/or carbamate group is distinct in its ability to halt the mechanism of the enzymes in the body. When the body receives these toxic pesticides, the enzymes will not function properly. Toophorm [7], used this fact as a preliminary test for toxicity to filter out the 2 groups of pesticides that are commonly used. The main feature of the basic test kit involves testing the degree of toxicity of the sample, whether it is an insecticide or toxic residue from plant cells and some chemicals that causes toxicity. These toxic substances also have the ability to reduce the performance of the enzyme. If the vegetables have pesticides/toxic residues, they can inhibit the cholinesterase enzyme. The enzyme cannot hydrolyze acetylcholine to choline. The high amount of acetylcholine remaining will give the high intensity of dark colours. In vegetables with higher pesticide residues, cholinesterase will be inhibited to a greater degree, resulting in acetylcholine remaining in large quantities and showing as a very dark colour in the kit. Toxicity of less than 50% is considered as safe for consumption. This technique was used in this research to determine the percentage reduction of toxicity after cleaning by measuring the toxicity of the residue before and after cleaning. The standard curve of the intensity of colour and degree of toxicity was made for this analysis [7].

Food safety is most important to consumers therefore ultrasonic cleaning of vegetables is a new alternative method to make food safe from excessive pesticide residues. The question that needs to be answered is why cleaning with ultrasonics is better than without ultrasonics, as well as how rapid is the reduction of pesticide residues by ultrasonic cleaning and soaking in water. By studying the kinetics of the reduction of pesticide residues, it is possible to compare the effectiveness of the cleaning. Such knowledge will help increase the safety for consumers, not only within this country but also Thailand's many import partners.

Materials and Methods

Vegetable treatment

Vegetables (coriander, kale, yard long bean and red chili) were purchased from a local market near Kasetsart University. These vegetables were dipped in the pesticide that has the common name "carbosulfan" which is in carbamate group (Sharpulators Co. Ltd.) in the ratio of pesticide 8 ml to water 6 L soaking for 20 min and then drained and air dried for 30 min.

Cleaning

Coriander, kale, yard long bean and red chili of 160, 220, 250 and 600 g, respectively [8], were used in the cleaning test of each replication. Each vegetable was immersed in 2 L of tap water with dimensions of 100×300×150 mm in the ultrasonic cleaner (Thailand Institute of Scientific and Technological Research, TISTR) and treated with and without ultrasonics for 0, 3, 7 and 10 min with 20, 25 and 30°C, operating at 60 kHz of frequency, 140W of power and 3 replications were made. Each vegetable was sample tested for degree of toxicity by GT-pesticide test kit [7].

Testing degree of toxicity by GT-pesticide test kit

Sampling 10 g of chopped vegetables and adding 10 ml of dichloromethane (solvent-1) and shaking 1 min were done to extract pesticides from vegetables, thereafter standing for 10-15 min. Then 1 ml sample extract was removed and supplemented with 1 ml 5% ethanol in water (solvent-2) and separated into two phases. The under layer is a sample extract and the top layer is a solvent-1. The solvent is evaporated with an air pump for about 3 min at 34°C in water bath. 0.25 ml sample extract was used, adding 0.5 ml cholinesterase (GT-1), after 10 min adding 0.25 ml acetylcholine (GT-2), stand for 60 min. Adding 1 ml hydroxylamine (GT-3), 0.5 ml hydrochloric acid (GT-4) and 0.5 ml ferric chloride (GT-5) was done respectively. The samples were centrifuged and the absorbance measured by a spectrophotometer at 540 nm wavelength and compared with standard curve (Fig. 1.) to determine the % inhibition (degree of toxicity) [7].

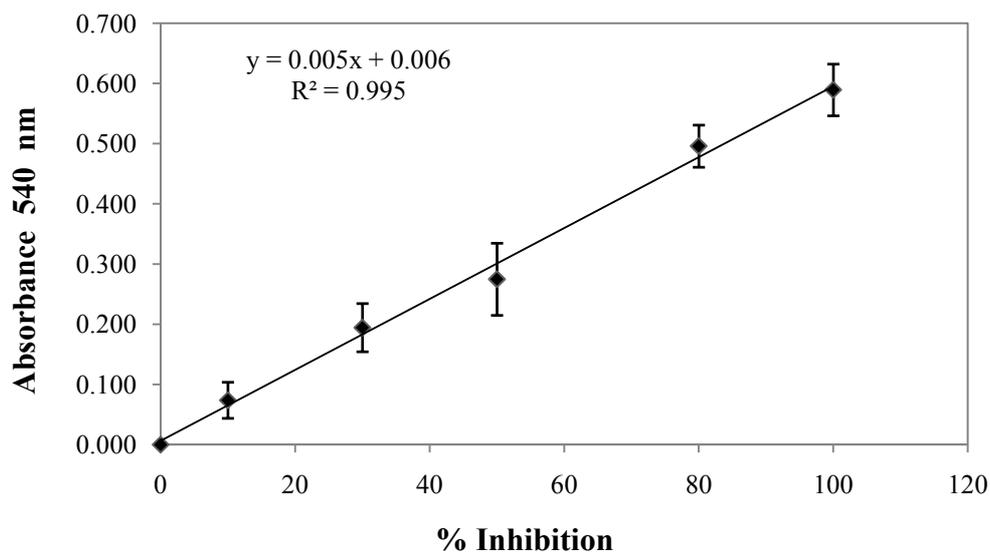


Figure 1. Standard curve of absorbance at 540 nm (colour intensity) and % inhibition (degree of toxicity).
(n=5)

Standard curve preparation

The standard curve of the intensity of colour and toxicity level of 0, 10, 30, 50, 80 and 100% was made by adding 1 ml of 5% ethanol in water and 0.5 ml cholinesterase (GT-1) in 6 tubes at 34°C in water bath, each tube representing a different level of toxicity. After 10 min, filling 0.25, 0.275, 0.325, 0.375, 0.45 and 0.5 ml acetylcholine (GT-2) for 0, 10, 30, 50, 80 and 100% of degree of toxicity (% inhibition), respectively. Standing 60 min before adding 1 ml hydroxylamine (GT-3), 0.5 ml hydrochloric acid (GT-4) and 0.5 ml ferric chloride (GT-5) was done respectively and 5 duplications were made. Next, the solution was centrifuged and measured for colour intensity in each tube by spectrophotometer at 540 nm. The standard curve was plotted between absorbance at 540 nm and % inhibition as shown in Figure 1.

Statistical analysis

All treatments were evaluated by mean ± standard deviation (SD) and the means between different experimental treatments were compared by Duncan’s multiple range test ($p < 0.05$) using SPSS program (version 12.0).

Results and Discussion

Cleaning with ultrasonic cleaner

Reductions of pesticide residues are described by the percentage of reduction of the initial inhibition of enzyme (degree of toxicity) and the final inhibition. The raw vegetables have a high amount of pesticide residues and thus high inhibition of enzyme and after cleaning the vegetables will have lower pesticide residue and less inhibition of enzyme. This reduction of pesticide residue was plotted with the time of cleaning (Fig. 2). For coriander and red chili, temperature and time of cleaning had influence on the reductions. Increased cleaning time, efficiency of cleaning is increased significantly ($p < 0.05$). Moreover, effect of cleaning time is not significant for 3 and 7 min of the all temperatures in kale and yard long bean. However, the performance at 10 min cleaning increased significantly ($p < 0.05$). Ultrasonic cleaning for coriander, kale, yard long bean and red chili reduced pesticide residues at 64, 63, 76 and 75% respectively.

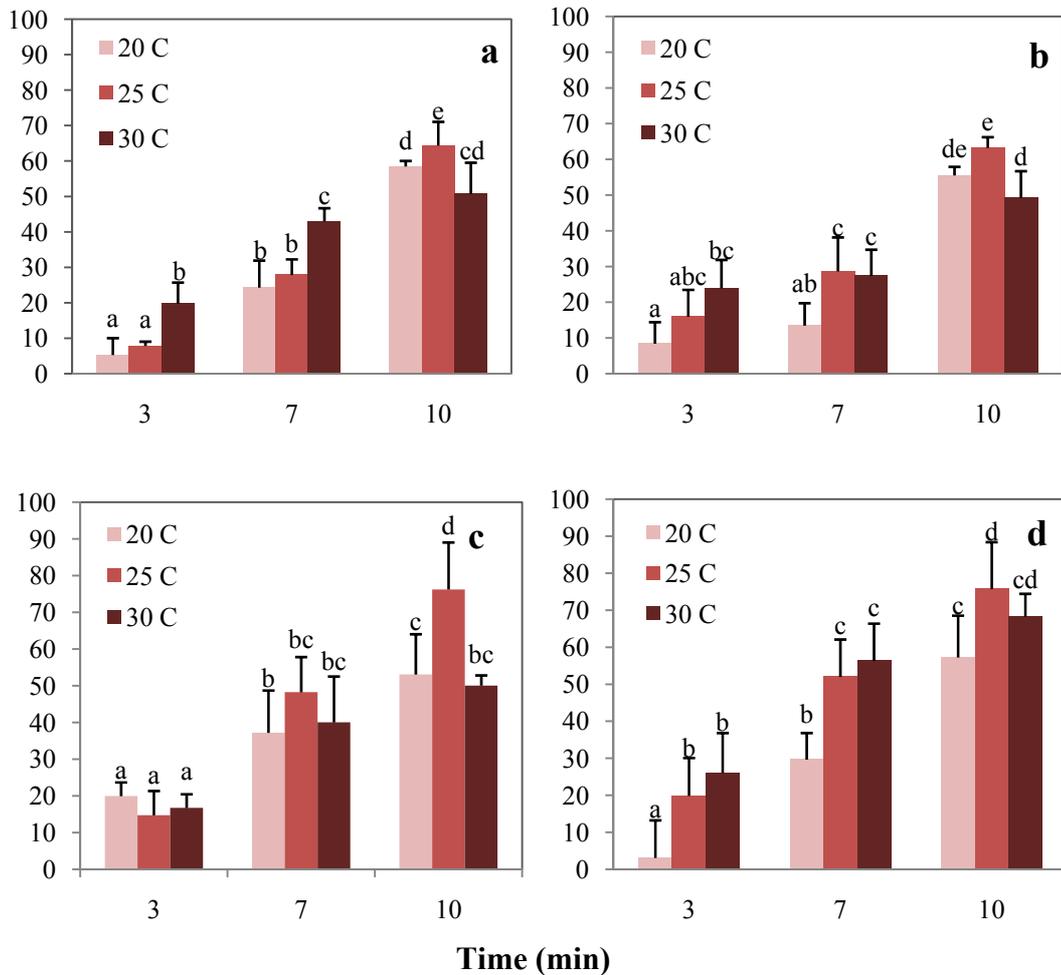


Figure 2. % reduction of the inhibition of enzymes (degree of toxicity) at various temperatures versus time of cleaning by ultrasonic cleaner.

Sonicated with ultrasonic at 60 kHz 140 W in (a) coriander, (b) kale, (c) yard long bean and (d) red chili, a-e were significantly different at $p < 0.05$

It is interesting to note that at 25°C for 10 min the reduction is highest and differs significantly to other temperatures ($p < 0.05$). Therefore, higher temperatures do not mean that the performance will be increased because temperature is the most important parameter to be regarded in optimizing cavitation intensity. Temperature changes result in changes in viscosity, the diffusion rate of dissolved gases, the solubility of gas in the liquid and vapour pressure and these have an effect on cavitation intensity [3]. When the temperature of the liquid increases, the number of bubbles also increases, therefore the cavitation is more intensive. However, as the temperature rises, the vapour pressure in the liquid is higher. The gas content in the bubbles increases, resulting in the strength of the cavitation collapse lessening. These two opposing reasons suggest that the appropriate temperature might occur when the cavitation is at maximum intensity [6]. This research suggests that the temperature of 25°C is the best condition for ultrasonic cleaning.

From the first-order reaction kinetics by Heldman *et al.* [9], the equation is given as:

$$-\frac{dC}{dt} = kC \tag{1}$$

Where C is the concentration, t is time, and k is the reaction rate constant. By integration, this equation becomes:

$$-\ln\left(\frac{C}{C_0}\right) = kt \tag{2}$$

Thus, according to this mathematical expression $\ln C$ vs. time, the linear function can be expected where the slope corresponds to k as shown in Figure 3.

The Arrhenius equation for effect of temperature can be shown as:

$$k = k_0 e^{-E_a / RT} \tag{3}$$

Where k_0 is the constant, E_a is the activation energy, R is the gas constant (1.987 cal/mole.K), and T is the absolute temperature (K).

When plotting the relationship of $\ln k$ and $1/T$ (1/K), the linear function can be seen where the slope corresponds to $-E_a/R$. Furthermore, the decimal reduction time (D) can be determined from the relationship of $\log C$ vs. time and is shown in Table 1.

Table 1. Effect of ultrasonic cleaning on reaction rate constant (k), Decimal reduction time (D) and activation energy of the reaction (E_a) of reduction of pesticide residue in four kinds of vegetables.

Vegetable	D (min)			$k \times 10^3$ (s ⁻¹)			$E_a \times 10^{-3}$ (cal/mole)
	20°C	25°C	30°C	20°C	25°C	30°C	
Coriander	26.2	22.3	32.6	1.38	1.62	1.20	2.462
Kale	28.2	22.8	34.6	1.20	1.53	0.98	3.443
Yard long bean	29.6	16.1	33.4	1.27	2.33	1.17	1.322
Red chili	25.2	17.8	20.0	1.48	2.17	1.97	5.021

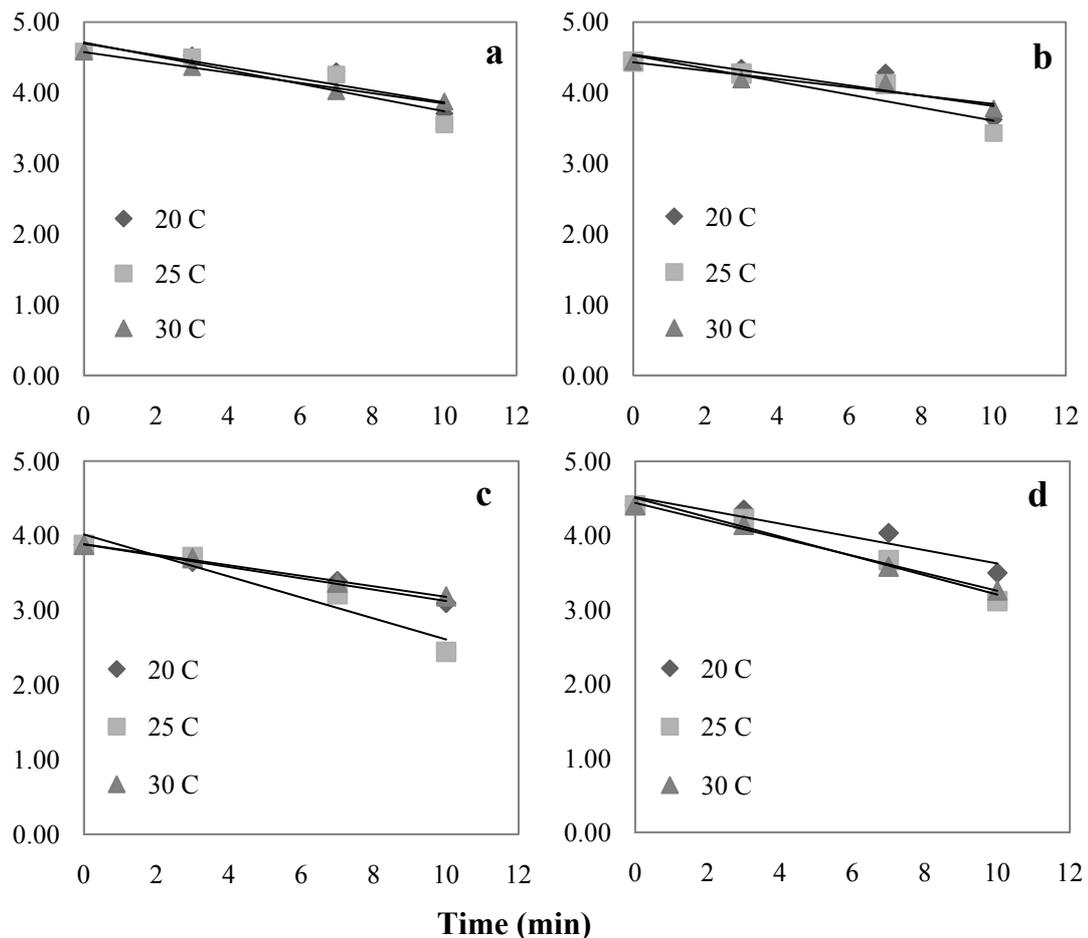


Figure 3. First-order kinetics of the reduction of pesticides by ultrasonic cleaning in (a) coriander, (b) kale, (c) yard long bean and (d) red chili.

The reaction rate constant (k) of ultrasonic cleaning was determined from the slope. A high k value means that reduction rate of pesticide residue is very fast. The decimal reduction time (D) means the time taken (min) to reduce 90% of pesticide residue by ultrasonic cleaning. A lower D value is good for cleaning because it can reduce pesticide residues faster and the activation energy of the reaction (E_a) indicates that temperature affects cleaning rate. It is recommended to use high activation energy for this type of cleaning. From Table 1 it can be seen that the pesticide residues are more greatly reduced at 25°C, as can be seen from the corresponding lowest D and highest k of the four kinds of vegetables. The D and k value at 25°C shows that reducing pesticide residues in yard long bean is higher than red chili, coriander and kale, respectively. It can be explained that rough surface of yard long bean increases cavitation bubbles, as opposed to the smooth surface of red chili as well as complex structure and crevices (limb and leaves) of coriander and kale which is hard to clean.

Cleaning without ultrasonic cleaner

The temperature of the four kinds of vegetables does not influence the cleaning significantly ($p \geq 0.05$). Although time has a significant influence when the time is longer, the ability to reduce pesticide residues increased ($p < 0.05$) as shown in Figure 4. For soaking in water for coriander, kale, yard long bean and red chili, the reduction of pesticide residues were 27, 29, 48 and 65% respectively.

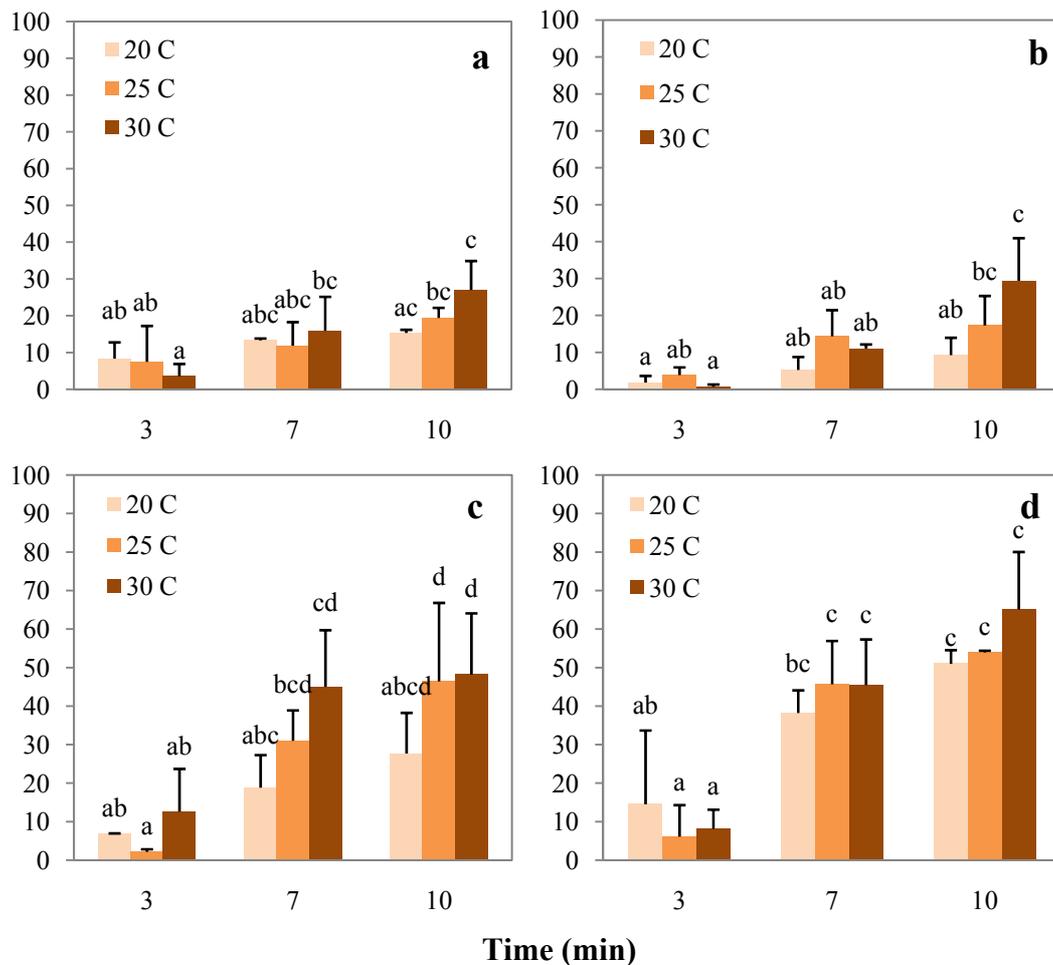


Figure 4. % reduction of the inhibition of enzyme at various temperatures compared with the % initial inhibition (before cleaning) versus time of cleaning by soaking in water for (a) coriander, (b) kale, (c) yard long bean and (d) red chili, a-d were significantly different at $p < 0.05$

Figure 5 shows the reduction rate constant (k) of cleaning without ultrasonic cleaner. The value of kinetic parameters for cleaning without ultrasonic cleaner is displayed in Table 2. It was found that 30°C is the optimum condition for cleaning of vegetables because high temperature will decrease the viscosity of water and will increase the discharge of pesticide residue, therefore, the reduction of pesticide is higher. The D and k value at 30°C in red chili shows the reduction of pesticide residues more than yard long bean, kale and coriander, respectively. The smooth surface and simple shape will allow pesticide residues and dirt to dissipate more easily than a complex shape like kale and coriander. For yard long bean surfaces which are seldom flat, instead being comprised of irregular surfaces, the removal of pesticide residue is more difficult. The high E_a value of kale indicates that it uses high activation energy for this cleaning and the effect of temperature for cleaning is significant.

Ultrasonic cleaning can reduce pesticide residues to a greater extent than water soaking; cavitation bubbles caused many small air bubbles in the liquid. These bubbles will grow, expand and regularly break out violently which causes mechanical energy as shockwaves as well as mixing the liquid medium to high heat and mass transfer and distribution within the very small pore of the solid surface. As far as liquid flow along with the eruption of cavitation bubbles near the asymmetric surface, this phenomena can reduce the pesticide residue and dirt better than cleaning without ultrasonic cleaner (by soaking in water).

Table 2. Effect of soaking vegetables in water on reaction rate constant (*k*), decimal reduction time (*D*) and activation energy of the reaction (*E_a*) for the reduction of pesticide residue in four kinds of vegetable.

Vegetable	<i>D</i> (min)			<i>k</i> x 10 ³ (s ⁻¹)			<i>E_a</i> x 10 ⁻³ (cal/mole)
	20°C	25°C	30°C	20°C	25°C	30°C	
Coriander	139.3	104.6	70.2	0.27	0.33	0.48	10.473
Kale	237.3	121.2	66.2	0.15	0.33	0.55	22.948
Yard long bean	76.9	33.3	32.4	0.50	1.20	1.30	16.931
Red chili	32.3	29.7	21.9	1.20	1.42	1.80	7.143

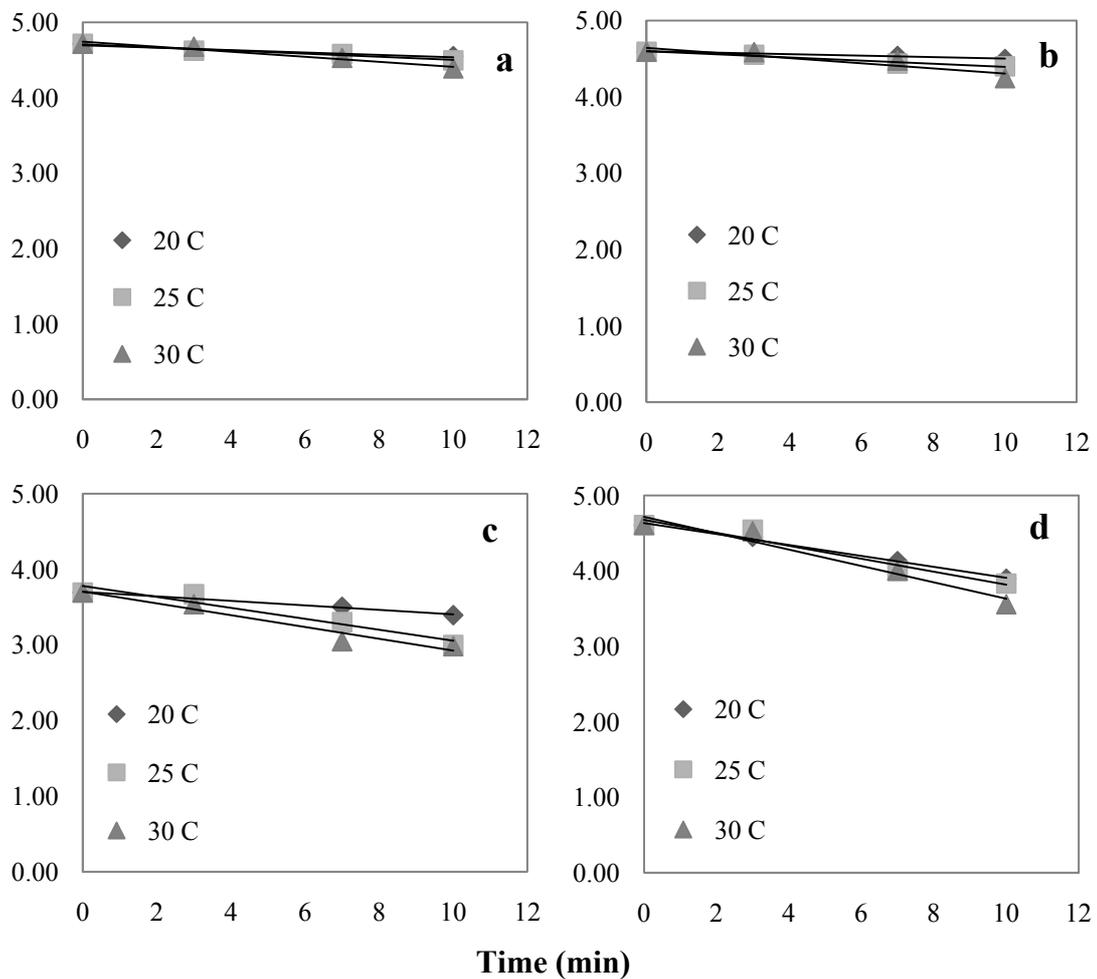


Figure 5. First-order kinetics of the reduction of pesticide residue for cleaning by soaking in water for (a) coriander, (b) kale, (c) yard long bean and (d) red chili.

The effect of cleaning temperature has a significant influence on ultrasonic cleaning but not a significant influence for soaking in water. However, results from *D*, *k* and *E_a* values show that 25°C for cleaning by ultrasonic cleaner and 30°C for cleaning by soaking in water are optimum for efficient cleaning of vegetables.

Conclusion

Ultrasonic cleaning of coriander, kale, yard long bean and red chili proved to be much more efficient than cleaning by soaking in water. The optimum temperatures for cleaning by ultrasonic cleaner are 25°C and 30°C for cleaning by soaking in water. The efficiency of the cleaning process can be deduced from the kinetics as to how fast is the reaction in vegetables by soaking and ultrasonic cleaning. The high reaction rate constant (k) of ultrasonic cleaning shows that reduction rate of pesticide residue is much faster. The low decimal reduction time (D) of ultrasonic cleaning means that it uses less time (min) to reduce 90% of pesticide residues and the high activation energy of the reaction (E_a) of ultrasonic cleaning indicates that temperature affects the high cleaning rate and thus high activation energy should be used for ultrasonic cleaning. Finally, the effect of long cleaning time such as 10 minutes has a significant effect on the reduction of pesticide residue ($p < 0.05$).

Acknowledgement

This research was supported by the Thailand Institute of Scientific and Technological Research, Ministry of Science and Technology under the corporation between TISTR and Academic Institute on Graduate Program Development.

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Nomenclature

C	the concentration
C_0	the initial concentration
D	the decimal reduction time (min)
E_a	the activation energy (cal/mol)
k	the reaction rate constant (s^{-1})
k_0	the constant
t	time
T	the absolute temperature (K)
R	the gas constant (1.987 cal/mole.K)