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Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var. *Crispa* L)

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ABSTRACT

Potential organic compatible sanitisers including electrolysed water (EW, 4 mg/L free available chlorine (FAC)), citric acid (0.6%), H_2O_2 (1%), and their combinations were applied on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var *crispa* L.) to evaluate their effects on microbiological safety, physicochemical parameters and sensory analysis (including raw sample and boiled sample). The combination of 1% H_2O_2 with 0.6% citric acid led to the highest reductions of microbial loads (2.26 log CFU/g for aerobic mesophilic count (AMC) and 1.28 log CFU/g for yeasts and moulds); however, it also caused the highest electrolyte leakage rate (3.11% vs. 0.91% for control). The combination of EW with 1% H_2O_2 achieved 1.69 and 0.96 log CFU/g reductions for AMC and yeasts and moulds, respectively with electrolyte leakage rate of 1.41%. In terms of the content of polyphenolic compounds, firmness, colour and raw material sensory analysis, there were no significant differences among different treatments, and between organic and conventional counterparts. The results suggest that 1% H_2O_2 combined with 4 mg/L EW is a promising approach for treating organic fresh-cut lettuce.

1. Introduction

With the consumers becoming increasingly concerned about their health, consumption of organic fresh vegetables has been increasing recently, due to less pesticide and other reasons (Grinder-Pedersen et al., 2003). In 2011, worldwide organic food sales were approximately 63 billion US dollars (Low, 2013). However, organic vegetables are generally grown with agricultural fertilisers including animal manure, resulting in a concern about the possible microbial contamination of the vegetables as the counterpart of conventional produce (Goodburn & Wallace, 2013; Ölmez & Kretzschmar, 2009). Thus it is important to find practical approaches to control microbial safety of both organic and conventional vegetables.

Some examples of the very recently fresh vegetable sanitisation techniques which have been used or studied for fresh produce are: different types of electrolysed water (EW) (Afari, Hung, King, & Hu, 2016; Yang, Feirtag, & Diez-Gonzalez, 2013; Zhang, Cao, Hung, & Li, 2016); 0.8%–5% H_2O_2 (Lopez-Galvez, Ragaert, Palermo, Eriksson, & Devlieghere, 2013; Lu, Joerger, & Wu, 2014; Van Haute, Tryland, Veys, & Sampers, 2015); 1%–2% organic acid (Tirawat, Phongpaichit, Benjakul, & Sumpavapol, 2016; Bermúdez-Aguirre & Barbosa-Cánovas, 2013); and also some combinations of these methods, such as combination of 100 mg/L EW with 1% citric acid (CA) (Park, Guo, Rahman, Ahn, & Oh, 2009), combination of 1% organic acids with 2% H_2O_2 (Lopez-Galvez et al., 2013). However, these techniques use too high concentration of sanitisers, which can't satisfy the requirement for processing fresh organic produce.

Currently, one common form of fresh lettuce is cut into bite-size pieces due to convenience, especially for those having busy lifestyle (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). One of the most common quality problems associated with fresh-cut lettuce is browning of the cut edges. During this process, the phenolic metabolism is altered in lettuce tissue which may result in the synthesis and accumulation of phenolic compounds (Vandekinderen et al., 2009). Thus, in addition to achieving microbial reductions, the effects of treatments on quality attributes such as firmness, electrolyte leakage rate (ECR) and colour; bioactive components such as phenolic compounds and sensory quality should also be considered in the evaluation of the efficacy of a sanitising process.

Due to strict regulations, for processing organic vegetables, only

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limited variety and concentration of synthetic sanitisers have been approved, such as low concentration of chlorine based sanitisers, organic acid produced by microbial fermentation of carbohydrate substances (as nonsynthetics), and H_2O_2 (no more than 1%) are allowed for sanitising organic food (CFR, 2014; Ölmez & Kretzschmar, 2009; Organic, 2013).

To our best knowledge, there is no systematic report of the combination of different potential organic sanitisers on fresh-cut lettuce. The aim of this study was to evaluate the effects of EW (4 mg/L FAC), 0.6% citric acid (CA), 1% H_2O_2, and their combinations on microbiological load, physicochemical quality and sensory property of organic and conventional fresh-cut loose-leaf lettuce. The results would contribute the application of some potential effective sanitising practices on the ever increasing organic food market especially fresh-cut vegetables.

2. Materials and methods

2.1. Sample preparation

Conventional and fresh organic lettuces were bought from a local farm in Singapore. The vegetables were transported to the laboratory and stored at 4 °C and used within 24 h of purchase. The three outermost leaves and the inner part of each lettuce were removed. Then after swiftly rinsed with tap water for 1 min to remove the soil on the leaves, a sterile kitchen knife was used to cut lettuce into pieces of 2–2.5 cm (Karaca & Velioglu, 2014).

Samples were immersed immediately in the following sanitiser solutions after prepared for 15 min (Alexandre, Brandão, & Silva, 2012; Karaca & Velioglu, 2014): (i) 0.6% CA (Sigma); (ii) EW (with 4 mg/L FAC, was obtained by the electrolysis of dilute sodium chloride solution using an electrolysed water generator (Hoshizaki ROX-10WB3-EW, Smitech (Asia) Pte Ltd, Singapore)); (iii) 1% H_2O_2 (prepared from a solution of 30–32% w/w, QRëC, Auckland, New Zealand); (iv) the combination of EW and 0.6% CA (EW + CA); (v) combination of EW and 1% H_2O_2 (EW + H_2O_2) (vi) the combination of 1% H_2O_2 and 0.6% CA (H_2O_2+CA). The ratio between mass of the vegetable sample and volume of solution was 50 g/L. Additional experiments using sterile deionised water (DI) washing were performed as control. The temperature of the solutions was 22±1 °C. The properties of the above seven sanitisers are shown in Table 1. The FAC was determined by a colorimetric method using a chlorine test kit and RQflex® 10 Reflectoquant® (Merck, Darmstadt, Germany) and pH was determined by using a pH meter (Metrohm Singapore Pte. Ltd, Singapore).

2.2. Microbiological analysis

After treated with sanitisers and DI, cut lettuce samples of 25 g each were homogenised in 225 mL sterile peptone water (0.1% (w/v), Oxoid, Cambridge, UK) for 2 min using a stomacher (IUL Instruments, Barcelona, Spain). Ten-fold dilution series prepared in 9 mL of sterile peptone saline solution were performed as needed and samples were plated in the appropriate culture media. For aerobic mesophilic count (AMC) and aerobic psychotropic count (APC), spread plating technique using plate count agar (PCA, Oxoid) was utilised followed by incubation at 37 °C for 48 h and at 7 °C for 10 days, respectively. For yeasts and moulds, potato dextrose agar (PDA, Oxoid), spread plating with incubation at 25 °C during 4 days was used. The sanitising treatments were replicated twice independently, and each sample was plated in duplicate at each analysis time point. All microbial counts were reported as log colony forming units per gram (log CFU/g) (Chong, Lai, & Yang, 2015; Seow, Ágoston, Phua, & Yuk, 2012).

Table 1
The concentration and pH of different washing solutions.

Solution	Concentration	pH
DIW	0	7.11 ± 0.13
CA	0.6% (w/v)	2.34 ± 0.01
EW	4 mg/L (FAC)	3.77 ± 0.18
H_2O_2	1% (w/v)	4.76 ± 0.23
EW + CA	4 mg/L + 0.6%	2.44 ± 0.05
EW + H_2O_2	4 mg/L + 1%	4.21 ± 0.23
H_2O_2+ CA	1% + 0.6%	2.52 ± 0.19

2.3. Physicochemical property analyses

2.3.1. Firmness, electrolyte leakage and colour measurement

Firmness of fresh-cut lettuce leaves was measured using a TA-XT2i Texture analyser (Stable Micro Systems Ltd, Godalming, UK) according to a previous method (Salgado, Pearlstein, Luo, & Feng, 2014) with slight modification. The press holder and the blade plunger were moved down at a velocity of 5 mm/s to1 cm below the bottom of the holder. The maximum cut force (MCF) was recorded using the Texture Expert Software (Nova-Tech International, Inc., Houston, TX, USA). These tests were conducted with six independent replicates for each group.

Tissue status was studied by measurement of the differences on the electrolyte leakage between samples treated using different sanitisers according to a previous report (Kim, Luo, Tao, Saftner, & Gross, 2005) with some modifications. Two samples per each treatment of fresh-cut lettuce of 10 g were disposed in a glass beaker covered tightly with aluminum-foil laminated paper and were immersed in 100 mL of distilled water, the electrical conductivity of which was measured by using a conductivity meter Horiba ES-14 (Horiba.Ltd, Kyoto, Japan). After 0.5 h, initial electrical conductivity was measured ($t_{0.5}$). Then, samples were stored at −20 °C for 24 h. Subsequently, samples were thawed overnight and electrical conductivity was measured when samples reached room temperature (conductivity t_{24}). Electrolyte leakage rate was expressed as percentage of total electrolytes released after 0.5 h. Every experiment was repeated four independent times.

$$\text{Electrolyte leakage}(\%) = \frac{\text{conductivity } t_{0.5} - \text{conductivity of distilled water}}{\text{conductivity } t_{24}}$$

For colour measurement, two pieces of cut lettuce leaves were withdrawn from each treatment and analysed using a Minolta Colorimeter CM-3500d (Konica Minolta, Inc., Tokyo, Japan). Hunter's colour values (L, a, b) were measured at 3 locations of each piece of lettuce and averaged for a total of 6 readings for each treatment. Overall colour difference was calculated by applying following formula (Pathare, Opara, & Al-Said, 2013): $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$, where, ΔE^*represents the overall colour difference. Standard white plate and black plate were used for instrument calibration. Each treatment was replicated independently three times.

2.3.2. Total phenolic contents

The extraction of total polyphenolic compounds was performed according to the methodology reported by Martínez-Sánchez, Marín, Llorach, Ferreres, and Gil (2006) with slightly modification. Freeze-dried leaves (0.5 g) were extracted in 10 mL extraction solution, consisting of 0.5 M HCl in methanol/Milli-Q water (80% v/v). The mixture was incubated overnight at 4 °C and then centrifuged with 12000 *g* for 20 min at 4 °C. Supernatant was recovered and filtered. The content of phenolic compounds in methanol extracts was determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). The method consisted of mixing 500 μL of the extract diluted in water with 500 μL of Folin-Ciocalteu's reagent. After 3 min of reaction, 1 mL of 1 M sodium carbonate (Na_2CO_3) was added. The tubes were mixed for 15 s and then allowed to stand for 60 min at 20 °C. Absorbance was measured at 725 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curves were daily prepared using gallic acid (3, 4, 5-trihydroxybenzoic acid). The analysis was repeated three independent times, every time with two parallel samples. The phenolic content was expressed in mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

2.4. Sensory analysis

Discrimination of samples according to the sensory quality of the product after washing with the sanitising agents and also the boiled counterpart (100 °C, 2 min) was carried out by a 13 member trained sensory panel. Sensory panel members were required to evaluate changes in green intensity, visual defects, crispness (for raw lettuce)/firmness (for boiled lettuce), off-odour and overall quality by using 9 to 1 point category test (Salgado et al., 2014) with modification.

For 'visual defects' and Off-odour, 1 means no countless defects/off odour, 3 a little, 5 quite some, 7 a lot, 9 countless defects; for 'crispness (fresh)' and overall quality: 1 extremely bad texture, 3 with some defects, 5 neutral, 7 good texture, 9 extremely good texture/firm. For overall quality attribute: 1 very poor, 3 quite poor, 5 moderate, 7 quite good, 9 very good. The different categories were obtained based on three rounds of training.

2.5. AFM analysis

Atomic force microscopy (TT-AFM, AFM workshop, Signal Hill, CA, USA) was used to perform bacterial morphological analysis. *Escherichia coli* ATCC 25922 was used as an indicator bacterium in all AFM experiments. For *E. coli* solution, 10 mL cultures of 24 h were centrifuged for 10 min (3600 *g*, 4 °C). Pellets were washed using 10 mL autoclaved DI, centrifuged and re-suspended in 10 mL autoclaved DI. During the experiments, 1 mL *E. coli* solution was mixed with 9 mL each treatment solution, after 30 s, 20 μL sample was dropped on a disinfected coupon and air dried. Vibrating mode was used and scan rate of 0.4 Hz and scan lines of 512 were set. NSC 11/no Al tips with resonance of 145e240 kHz and force constant of 25–95 N/m were used. AFM images were analysed off-line by using Gwyddion software (AFM workshop). Qualitative data were obtained through analysing bacterial length, height and width from images using the software.

2.6. Statistical analysis

Results were reported as means ± standard deviations. Analysis of variance (ANOVA) and Duncan's test were performed to examine the differences between groups by SAS software (SAS Institute Inc., Cary, NC, USA). Comparisons with *P* value less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Microbiological analysis

Fig. 1 shows the surviving population of AMC (Fig. 1A), APC (Fig. 1B), and yeasts and moulds (Fig. 1C) on fresh-cut conventional and organic lettuce before and after washing with DI, CA, EW, EW + CA, EW + H_2O_2, H_2O_2+CA, and H_2O_2. As can be seen from Fig. 1 A–C, there was no significant difference among different types of lettuce. The similar result also can be seen for the comparison of AMC and APC of the lettuce with same treatment. For different treatments, none of the single antimicrobial agents (except H_2O_2) had any significant sanitising effect compared to control. The populations of AMC were 5.63, 5.58, 5.65, and 4.61 log CFU/g for the control, DI, CA, EW and H_2O_2 treatments, respectively, while the population of yeasts and moulds were 4.63, 4.74, 4.60 and 3.78, respectively (Fig. 1). The H_2O_2 based sanitiser achieved significant microorganism reduction. The greatest reduction was achieved by the treatment of H_2O_2 combined with CA, being around 2.26 log CFU/g of AMC and 1.28 log CFU/g for yeasts and moulds. The combination of 4 mg/L EW with H_2O_2 had the second best sanitising effect, causing 1.69 and 0.96 log CFU/g reductions of AMC and yeasts and moulds, respectively. In our study, the combination of EW and CA had the lowest microbial reduction among the combined methods, only resulted in reductions of AMC and yeasts and moulds counts of about 0.76 and 0.77 log CFU/g, respectively (Fig. 1A and C), even less than H_2O_2 used alone (0.97 and 0.95 log CFU/g reductions, respectively).

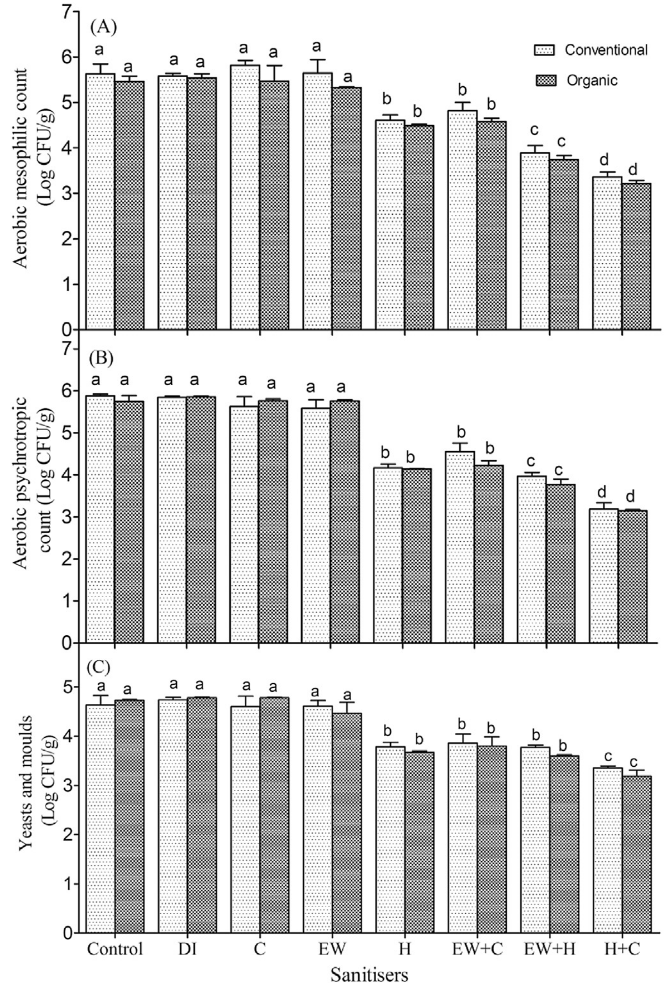

Fig. 1. Surviving population of aerobic mesophilic count (A), aerobic psychrophilic count (B) and yeasts and moulds(C) on fresh-cut conventional and organic lettuce after washing with DI, 0.6% CA, 4 mg/L EW, 1% H_2O_2 (H), combination of 4 mg/L EW with 0.6% CA (EW + C), combination of 4 mg/L EW with 1% H_2O_2 (EW + H), combination of 1% H_2O_2 with 0.6% CA (H + C). Control is the one without wash (No wash). *Means within each group with different lowercase letters are significantly different among different treatment ($P < 0.05$).

Table 2
Comparison of the dimensions of bacteria with different treatment by using AFM.

Treatments	DI	H_2O_2	H_2O_2 + EW	H_2O_2 + CA
Length (L/μm)	2.58 ± 0.30^a	1.98 ± 0.45^{ab}	1.89 ± 0.11^b	1.15 ± 0.41^c
Width (W/μm)	1.36 ± 0.13^a	1.08 ± 0.09^b	0.79 ± 0.17^c	0.95 ± 0.07^{bc}
Height (Z/nm)	164.2 ± 31.8^a	134.5 ± 32.2^b	63.1 ± 12.3^b	86.2 ± 15.4^b

* Values in the same row with different superscript letters indicate significant differences by the Duncan's multiple range test ($P < 0.05$).

In the current study, the high initial AMC (unwashed) (5.63 log CFU/g) and yeasts and moulds (4.63 log CFU/g) of conventional fresh-cut lettuce agrees with a previous report (Salgado et al., 2014). Several possible factors contribute to these results: the unfold local lettuce leaf pattern which can provide a large exposed surface area and folds for microbial attachment and biofilm growth (Bermúdez-Aguirre & Barbosa-Cánovas, 2013), the increasing of exposed cut-surface and the introduction of additional organic matters caused by cut (Abadias, Usall, Anguera, Solsona, & Viñas, 2008), and the general high atmosphere temperatures in Singapore (Fu, Reineke, Chirtel, & VanPelt, 2008). These three reasons can also explain why the single antimicrobial agents (except 1% H_2O_2), such as 4 mg/L EW and 0.6% CA nearly had no reduction of microbial populations compared with control. For EW water, our result is consistent with previous reports (Keskinen, Burke, & Annous, 2009; Tan et al., 2015), that, the disinfected concentration of chlorine based sanitisers is 50–200 mg/L. In our study the FAC was only 4 mg/L. Park et al. (2009) evaluated the effect of 1% CA and combined effect of EW (100 mg/L) with 1% CA on grain, and found an approximately 2 log CFU/g reduction for *Bacillus Cereus* cells and spores. However, in another report, CA 3% was used to inactivate *E. coli* O157:H7 on fresh-cut lettuce, no inactivation was achieved after 15 min (Bermúdez-Aguirre & Barbosa-Cánovas, 2013). Our result agreed with this study. Beside the three reasons above, additional reason could be that organic acids cause loss of the ‘active substance’ when inactivation of microorganisms is needed in the presence of organic compounds (Hemond, 1990).

For the organic unwashed fresh-cut lettuce, the AMC, and yeasts and moulds were 5.46 and 4.72 log CFU/g, respectively (Fig. 1 A). It was very similar to that of the conventional lettuce. This agrees with previous study on the comparison of microbiological quality of organic and conventional vegetables in other countries (Oliveira et al., 2010; Maffei, de Arruda Silveira, & Catanozi, 2013). The AMC was very similar to that of APC. This result is supported by previous reports on lettuce because most of the microorganisms present in mesophilic conditions are also able to grow at refrigerated storage temperatures (Abadias et al., 2008; Oliveira et al., 2010; Seow et al., 2012).

Some combined groups achieved more sanitising effects than that of single one. The greatest reduction was achieved with the treatment of combination of 1% H_2O_2 with 0.6% CA. This result is consistent with previous study (Lopez-Galvez et al., 2013), who found that a larger reduction was obtained on lettuce treated with a combination of organic acid and 2% H_2O_2 than 2% H_2O_2 alone. In our study, after sanitising by these H_2O_2 based sanitisers, the population of aerobic bacteria in the lettuce leaves satisfied the regulation in Singapore that the aerobic bacterial count for ready-to-eat food should not be more than 5.0 log CFU/g (AVA, 2005). As only H_2O_2 based sanitisers attained significant bacteria reduction, the following physicochemical property analysis, sensory analysis and AFM analysis were only include the H_2O_2 based sanitisers' treatment and control (DI).

3.2. Physicochemical property analysis

3.2.1. Firmness, electrolyte leakage rate and colour

The larger MCF (maximum cut force) values, indicates a loss of turgor in the treated samples (Salgado et al., 2014). The effects of different processing conditions on the textural changes of conventional and organic lettuce are shown in Table 3. The results show that there were no significantly difference between different treatments and different sample groups. For the conventional lettuce, the value of MCF was from 14.9 to 18.8 N, and the organic one was from 13.2 to 14.1 N. A similar trend was found in Alexandre et al.'s study (2012) that effect of H_2O_2 at 1% on watercress's texture was equivalent to water-washing.

Tissue status was studied by measurement of the electrolyte leakage rate (ECR), lager ECR means more tissue damage (Kim et al., 2005; Salgado et al., 2014). The results of ECR in conventional and organic lettuce after different treatments are shown in Table 3. For each treatment, there was nearly no significant difference of ECR between conventional and organic lettuce ($P < 0.05$). The ECR of control of conventional and organic lettuce were 0.91% and 0.93%, respectively, which were increased after sanitising treatment. The combination of 1% H_2O_2 with 0.6% CA caused highest electrolyte leakage rate (3.11% and 3.43% for conventional and organic lettuce, respectively), followed by combination of EW with 1% H_2O_2 (1.41% and 1.54% for conventional and organic lettuce, respectively). This

Table 3
Effects of different treatments on firmness, electrolyte leakage rate, colour and phenolic compounds of conventional and organic lettuce.

Parameter			Treatment			
			DI	H_2O_2	H_2O_2 + CA	H_2O_2 + EW
Firmness (N)		Conventional	14.9 ± 2.89^{Aa}	14.7 ± 0.99^{Aa}	16.9 ± 3.03^{Aa}	18.8 ± 1.63^{Ba}
		Organic	13.2 ± 1.84^{Aa}	14.0 ± 2.10^{Aa}	14.1 ± 1.87^{Aa}	13.2 ± 2.00^{Aa}
Electrolyte leakage (%)		Conventional	0.91 ± 0.03^{Ac}	1.43 ± 0.02^{Ab}	3.11 ± 0.58^{Aa}	1.41 ± 0.11^{Ab}
		Organic	0.93 ± 0.04^{Ac}	1.37 ± 0.13^{Ab}	3.43 ± 0.29^{Aa}	1.54 ± 0.10^{Ab}
Colour	L^*	Conventional	45.02 ± 7.76^{Aa}	47.02 ± 4.39^{Aa}	48.11 ± 5.56^{Aa}	48.18 ± 3.89^{Aa}
		Organic	50.83 ± 3.14^{Aa}	46.91 ± 5.61^{Aa}	49.18 ± 4.52^{Aa}	47.11 ± 6.21^{Aa}
	a^*	Conventional	−9.21 ± 1.1^{Aa}	−9.92 ± 0.86^{Aa}	−9.13 ± 1.26^{Aa}	−8.88 ± 0.7^{Aa}
		Organic	−9.83 ± 0.67^{Aa}	−9.54 ± 1.0^{Aa}	−9.89 ± 1.06^{Aa}	−10.13 ± 0.51^{Ab}
	b^*	Conventional	28.38 ± 3.61^{Aa}	30.12 ± 2.03^{Aa}	29.38 ± 5.56^{Aa}	28.90 ± 3.84^{Aa}
		Organic	30.16 ± 2.28^{Aa}	30.25 ± 3.2^{Aa}	30.47 ± 3.83^{Aa}	31.14 ± 3.98^{Aa}
	ΔE	Conventional	54.06 ± 8.32^{Ba}	56.75 ± 4.43^{Aa}	57.17 ± 5.10^{Aa}	56.91 ± 5.58^{Aa}
		Organic	59.99 ± 2.54^{Aa}	56.68 ± 5.98^{Aa}	58.73 ± 5.36^{Aa}	58.49 ± 5.23^{Aa}
Total phenolic content (mg of gallic acid equivalents (GAE) per 100 g of FW)		Conventional	115.72 ± 9.14^{Aa}	133.33 ± 13.34^{Aa}	127.57 ± 25.11^{Aa}	130.83 ± 23.60^{Aa}
		Organic	110.11 ± 12.44^{Aa}	119.26 ± 2.90^{Aa}	107.83 ± 13.55^{Aa}	115.64 ± 4.01^{Aa}

* Means for same treatments of different groups with different capital letters are significantly different ($P < 0.05$). Within column, means with different small case letters are significantly different ($P < 0.05$) among different treatments.

Table 4
Effects of different treatments on sensory analysis of conventional and organic lettuce.

Parameters			Treatment			
			DI	H_2O_2	H_2O_2 + CA	H_2O_2 + EW
Raw lettuce	Off-odour	Conventional	1.6 ± 1.1^{Aa}	1.9 ± 1.6^{Aa}	1.5 ± 0.5^{Aa}	1.5 ± 0.5^{Aa}
		Organic	1.2 ± 0.4^{Aa}	1.3 ± 0.5^{Aa}	1.5 ± 0.6^{Aa}	1.6 ± 0.6^{Aa}
	Visual defects	Conventional	2.9 ± 2.0^{Aa}	2.9 ± 1.8^{Aa}	1.9 ± 1.1^{Aa}	2.5 ± 1.3^{Aa}
		Organic	2.2 ± 1.2^{Aab}	1.5 ± 0.5^{Bb}	2.2 ± 1.4^{Aab}	3.0 ± 2.4^{Aa}
	Crispness	Conventional	7.2 ± 1.5^{Aa}	7.3 ± 1.4^{Aa}	7.5 ± 1.1^{Aa}	6.6 ± 1.7^{Aa}
		Organic	7.5 ± 1.4^{Aa}	7.7 ± 1.1^{Aa}	7.6 ± 1.0^{Aa}	7.4 ± 1.7^{Aa}
	Overall quality	Conventional	7.2 ± 1.7^{Aa}	7.2 ± 1.2^{Aa}	7.6 ± 1.1^{Aa}	7.3 ± 1.1^{Aa}
		Organic	8.1 ± 0.9^{Aa}	8.3 ± 0.7^{Ba}	7.7 ± 1.2^{Aa}	7.7 ± 1.6^{Aa}
Boiled lettuce	Off-odour	Conventional	2.2 ± 1.7^{Aa}	1.8 ± 1.3^{Aa}	1.9 ± 1.8^{Aa}	2.0 ± 1.8^{Aa}
		Organic	2.2 ± 1.2^{Aa}	1.5 ± 0.6^{Aa}	1.5 ± 0.6^{Aa}	1.5 ± 0.6^{Aa}
	Visual defects	Conventional	1.5 ± 0.9^{Ab}	1.7 ± 0.9^{Ab}	1.5 ± 1.9^{Ab}	4.7 ± 2.1^{Aa}
		Organic	1.3 ± 0.5^{Ab}	1.5 ± 1.1^{Ab}	1.7 ± 1.3^{Ab}	4.5 ± 2.0^{Aa}
	Overall quality	Conventional	8.1 ± 1.1^{Aa}	7.9 ± 1.4^{Aa}	7.5 ± 1.7^{Aa}	5.0 ± 1.6^{Ab}
		Organic	8.0 ± 1.6^{Aa}	8.0 ± 1.4^{Aa}	7.8 ± 1.5^{Aa}	5.8 ± 2.0^{Ab}

* Values for same treatments of different groups of lettuce with different capital letters are significantly different ($P < 0.05$). Within same group of lettuce, values with different small case letters are significantly different ($P < 0.05$) among different treatments.

indicates that the H_2O_2 based sanitisers can cause some tissue damages. The similar trend was also found in Lopez et al.'s study (2013) by using fresh-cut iceberg treated with other H_2O_2 based sanitisers.

The results of colour (L*, a*, b*, ΔE) of conventional and organic lettuce with different treatments are shown in Table 2. There was no significant difference between the H_2O_2 based sanitisers and control, no matter it was conventional or organic lettuce. It was reported that H_2O_2 treatment induced development of browning in fresh-cut lettuce (Ölmez & Kretzschmar, 2009; Ramos et al., 2013), which can be readily followed by measuring a* values (Pathare et al. 2013). However, only high concentration of H_2O_2 (5%) produced negative impact on products' colour while no such effect treated with low concentration (1%) (Alexandre et al., 2012).

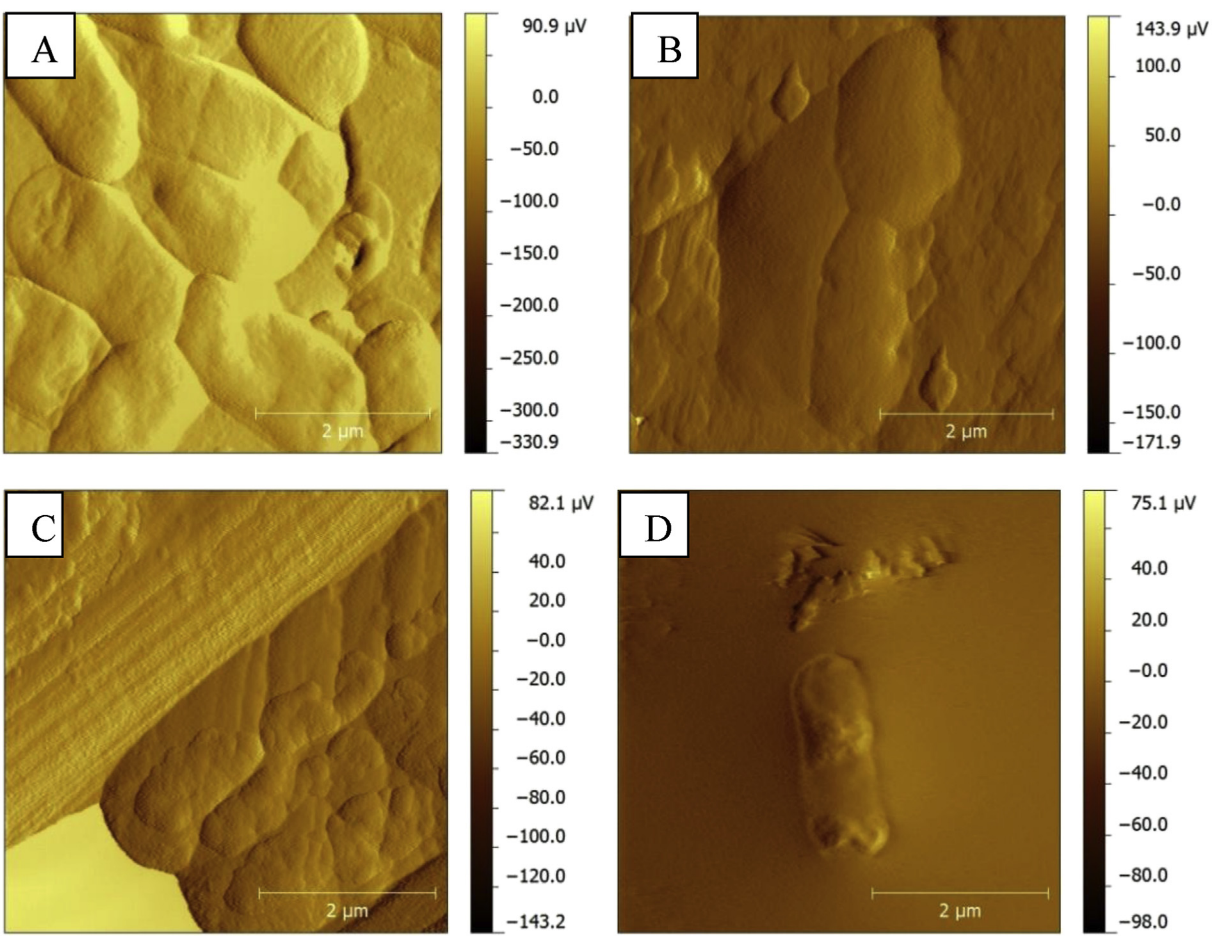

Fig. 2. Effects of treatment on *E. coli* ATCC 25922 bacterial cells imaged by atomic force microscopy. (A) control group (DI); (B) treated with 1% H_2O_2; (C) treated with combination of 4 mg/L EW with 1% H2O2; (D) treated with combination of 1% H_2O_2 with 0.6% CA.

3.2.2. Polyphenolic compounds

The phenolic content of fruit and vegetables influences both the organoleptic and nutritional qualities (Martínez-Sánchez et al., 2006). The total phenolic contents of conventional and organic lettuce with different treatments in our study are shown in Table 3. No obvious difference was observed between different treatments when compared to the control. In our study the initial value of conventional lettuce washed with DI was 124.42 mg/100 g EW, which agrees with a previous report (Martínez-Sánchez et al., 2006).

These results indicate that different sanitising treatments do not significantly affect the total phenolic contents of both the conventional and organic lettuce. There are some reports that organic vegetables contain more phenolic compounds compared to conventional counterparts (Grinder-Pedersen et al., 2003; Sobieralski, Siwulski, & Sas-Golak, 2013) due to the growth environment of organic fertilisers, which results in producing more carbon compounds, more sugars, phenolic compounds and ascorbic acid (Sobieralski et al., 2013). For the conventional vegetables, mineral fertilisers used, produce more nitrogen compounds, including amino acids, peptides, proteins and alkaloids. However, in our study the value of conventional lettuce was nearly the same amount as that of organic lettuce for each treatment. The possible reason is that this kind of organic lettuce was grown in a green house in Singapore, which has less sunlight than that grow on farms (Grinder-Pedersen et al., 2003).

3.3. Sensory analysis

Lettuce has no external protective tissue, and processes like cutting and sanitising expose its tissues to air and sanitisers. Lettuce is affected detrimentally by most of these chemical compounds when used beyond certain critical concentrations, leading to browning, tissue damage, colour changes, water segregation, and overall poor appearance (Ramos et al., 2013). As lettuce is not only consumed raw but also consumed after being boiled, thus both the raw and boiled lettuce were assessed by the sensory panel. Sensory results of raw and boiled conventional and organic lettuce samples after all four sanitisation treatments are shown in Table 4.

For raw lettuce, the scores of overall quality attribute of all treatments were above 7, which were quite acceptable by the sensory panel, for both conventional and organic lettuce. Although for green intensity and visual defects, there were some slight differences of the scores for different treatments, they were still at the same rating (around 3). No significant differences were observed of off-odours and crispness score among different treatments between conventional and organic lettuce. This agrees with a previous study (Lopez-Galvez et al., 2013).

For boiled lettuce, there was nearly no significant difference of all the indexes among all the treatments except the visual defects of combination treatment of H_2O_2 with 4 mg/L EW, which was one rating worse than others. According to our results, the overall quality attribute of all treatments was acceptable.

3.4. AFM analysis

Fig. 2A–D shows representative AFM images of *E. coli* after inactivation by 1% H_2O_2, combination of 4 mg/L EW with 1% H_2O_2, combination of 1% H_2O_2 with 0.6% CA and DI. *E. coli* treated with DI were rod shaped and kept intact. However, after treatment with H_2O_2 based sanitisers, the damage to the structures was seen very clearly, such as shrunk and fracture, especially for the group treated with combination of 1% H_2O_2 with 0.6% CA, which showed a significant morphological change. Cho, Kim, Kim, Yoon, and Kim (2010) also found a significant morphological change with ozone-treated *E. coli* by using transmission electron microscopy (TEM). Apart from imaging a microorganism, AFM has another advantage that it can provide characteristic parameters such as length, width, and height of the bacteria (Yang & Wang, 2008). These parameters of the *E. coli* from AFM results are shown in Table 1. The length, width and height of *E. coli* were 2.58 μm, 1.36 μm, and 164.2 nm, respectively. After sanitising treatment all the parameters were decreased, which agrees with the image results. The possible reason is that the H_2O_2 acts as an oxidant by producing hydroxyl free radicals (•OH) which attacks essential cell components, including lipids, proteins, and DNA. When existed with acid, it can produce more •OH (Back, Ha, & Kang, 2014).

4. Conclusion

Different potential sanitisers including H_2O_2 based sanitiser systems, such as 1% H_2O_2, 1% H_2O_2 combined with 4 mg/L EW or 0.6% CA, effectively inactivated microbial cells of fresh-cut lettuce while maintaining its sensory quality. The combination of 1% H_2O_2 with 0.6% citric acid provided the highest reductions of microbial loads, resulting ina reduction of 2.26 log CFU/g for AMC and 1.28 log CFU/g for yeasts and moulds; however, this treatment also caused the highest electrolyte leakage rate. The combination of EW with 1% H_2O_2 achieved 1.69 and 0.96 log CFU/g reductions for AMC and yeasts and moulds, respectively with electrolyte leakage rate of 1.41%. The combination of 1% H_2O_2 with 4 mg/L EW efficiently reduced microbial loads, while didn't compromise the quality and sensory properties of lettuce. Thus, this combination is a potential promising approach for treating fresh-cut organic lettuce.

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