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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