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Nonthermal pasteurization of Pitaya (*Hylocereus polyrhizus*) juice using the hurdle concept

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Abstract

Red pitaya juice (RPJ) was subjected to UV-C irradiation and the potential of UV as a pasteurization tool for reducing microbial load in pitaya juice was evaluated. Effectiveness of the hurdle concept, i.e. addition of citric acid (CA) and dimethyl dicarbonate (DMDC) was also studied. Total plate counts (TPC) and yeast and mould counts (YMC) achieved 2.43 $\log_{10}$ and 2.7 $\log_{10}$ reductions respectively after exposure to UV irradiation. Addition of the CA (0.5 – 2.0%) and dimethyl dicarbonate (DMDC) (5 – 20 µL/100mL) to pitaya juice reduced the microbial loads, with 1.5% CA and 15 µL/100mL DMDC being the most effective concentrations. Addition of CA and DMDC into RPJ prior to UV treatment achieved significantly higher microbial reduction compared to UV alone, which were 4.12 $\log_{10}$ and 4.14 $\log_{10}$ reductions for TPC and for YMC, respectively.

Introduction

Red pitaya is an attractive fruit with many kiwi-like seeds scattered in the bright purplish-red coloured pulp. The flesh and peel of red pitaya contains betacyanin pigments (betalains) which contributes to the fruit's unique colour and high antioxidative activity (Wybraniec and Mizrahi, 2002; Wu *et al*., 2006; Jamilah *et al*., 2011). Despite these properties, the naturally attractive colour of the juice is not stable to processing conditions involving heat. Preliminary work indicated that thermal treatment causes darkening (loss of brightness) of red pitaya juice (RPJ) as well as destruction of its mild taste. Since colour and taste are important quality indicators for juices, selection of proper processing methods to retain the juice quality is necessary.

Non-thermal technologies such as pulsed electric fields (PEF), ultraviolet (UV) light and high pressure processing have been applied in food processing as an alternative to thermal processing (Guerrero-Beltrán and Barbosa-Cánovas, 2004). The absence of heat in non-thermal technology produces better quality food products such as fruit juices (Noranizan and Benchamaporn, 2007). Fruit juices treated with UV has long been approved by the American government for commercial production (Anon, 2000; USFDA, 2000). However, application of UV for pasteurisation of RPJ may be limited by the high colour intensity of RPJ and the high viscosity of this mucilage-rich juice. These properties may result in limited penetration, hence, the reduced efficiency of microbial inhibition by UV light. In consideration of these aspects, it is important to ensure low microbial load prior to UV treatment by combining with other treatments, i.e. hurdle concept.

Hurdle concept, i.e. addition of CA and dimethyl dicarbonate (DMDC), has shown promising results of microbial reduction in fruit juices (Bizri and Wahem, 1994; Williams *et al*., 2006; Fisher and Golden, 2007). Addition of CA increases the acidity of foods and this method is one of the oldest preservation methods which are still being applied in the food industry. DMDC is commonly used as yeast inhibitor in the wine industry, but its effectiveness as yeast inhibitor in fruit juices is scarcely reported. Also, limited studies were reported on bacterial pathogens reduction by DMDC (Williams *et al*., 2006; Fisher and Golden, 2007). Hence, the purpose of this study was to subject RPJ to UV-C and to evaluate its effectiveness on the microbiological quality of RPJ. The effects of citric acid and dimethyl dicarbonate for microbial inactivation were also evaluated.

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Materials and Methods

Experimental design

This work was divided into four sections involving ultraviolet irradiation, addition of antimicrobial agents (citric acid and dimethyl dicarbonate), and application of the hurdle concept in extending shelf life of RPJ. The first section involved UV irradiation and evaluation of microbial load reduction in the juice after irradiation. In the second section, the effect of different concentrations of citric acid on microbial reduction was also evaluated and the most effective concentration was then selected to combine with UV. Citric acid was added into the juice to further reduce pH of juice for preservation purposes. In the third section, dimethyl dicarbonate (DMDC), a processing aid was added into the prepared juice at various permitted concentrations. The concentration that reduced the most microbial number was then selected to be combined with UV. The fourth section was determining the effectiveness of selected treatments when applied as a combination of non-thermal microbial reduction technique (UV, citric acid and DMDC).

Juice preparation

Red fleshed pitaya (*Hylocereus polyrhizus*) fruit at commercial maturity were purchased from a local market and were stored at 4°C before juice extraction. The fruits were washed, peeled, weighed and cut into slices manually. Part of the mucilaginous material and seeds were separated using a paddle depulper (Bonina., Brazil) and the mashed pulp was collected before further being filtered to obtain juice. The extracted juice was stored in closed container at 4°C before further processing. Fresh RPJ samples (control) were immediately analysed.

Treatment of juice

a) Ultraviolet irradiation

Five litres of RPJ was placed in the stainless steel pot and was treated with ultraviolet irradiation using the *CiderSure* 3500-B ultraviolet pasteurizer (Macedon, New York) that holds (in a vertical position) eight low pressure lamps which emit light at the 254 nm wavelength. Figure 1 illustrates the layout of basic components in the UV pasteurizer. The treatment chamber has a gap of 0.064 cm with dead volume of tube approximately 180 ml. The flow rate is automatically adjusted depending on light transmission properties of the treated juice and is controlled by a positive displacement vane pump (56C frame motor, PROCON) equipped with a UV sensor and a flow rate monitoring system. The irradiated RPJ was then collected into clean, sterilized glass bottles and capped with sterilize cap bottles. The juices were stored at 4 ± 1ºC until analysis. Three samples were prepared for each treatment.

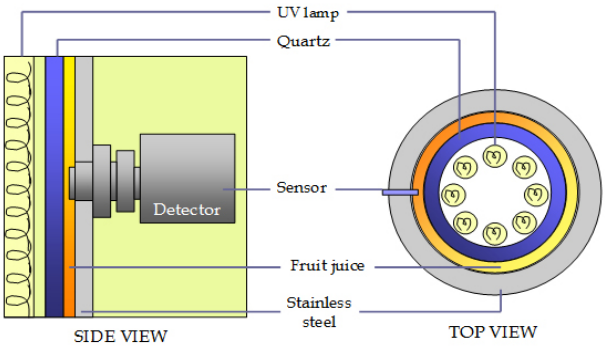

Figure 1. Layout of basic components in the *CiderSure* 3500 UV pasteurizer (adapted from Mohd Adzahan, 2006)

b) Addition of citric acid

Five litres of RPJ was separated into five different sterilized glass bottles and added with citric acid at various concentrations (0, 0.5, 1.0, 1.5 and 2.0 %) as described by Mosqueda-Melgar *et al*. (2008). The juice was kept at 4 ± 1ºC until analysis.

c) Addition of dimethyl dicarbonate (DMDC)

Five litres of RPJ in clean and sanitized stainless steel containers were added with various concentrations of DMDC (0, 5, 10, 15 and 20 µL/100mL). The juice was then filled into sterilized glass bottles and kept at 4 ± 1ºC until analysis.

d) Combined treatments

Based on results of microbial counts in RPJ, the most effective concentrations of citric acid and DMDC were chosen for the combination study. Five litres of juice were treated with citric acid and DMDC at the selected concentrations and subsequently exposed to ultraviolet irradiation. For simplicity, the combined non-thermal treatment sequence was given the term NTCA+DMDC+UV. Addition of citric acid and DMDC were done prior to UV irradiation to increase the efficiency of UV irradiation by reducing microbial counts in the juice prior to UV exposure.

Microbiological analyses

To evaluate total plate count (TPC), 10 grams of RPJ was added into 90 ml of 0.1% peptone water in the stomacher bag. The samples were homogenized using a stomacher for about 30 seconds. The serial dilution was prepared as diluents in duplicates and spread on Plate Count Agar (Oxoid CM 0463 standard PCA, Basingstoke). The plates were then incubated at 37ºC for 24 hours.

For yeasts and moulds, serial dilution for

enumeration of yeast and moulds were prepared as diluents. Dichloran Rose Bengal Chloramphenicol agar (Oxoid CM 0727 DRBC Agar Base, Basingstoke) was spread over duplicate plates for each dilution. The plates were then incubated at 30 ºC for 5 days. Microbial counts were done only for plates containing 30-300 colonies after incubation. Results were expressed as colony forming units (CFU/mL) of juice.

Titratable acidity and pH

Twenty millilitres of RPJ was accurately weighed into 50 mL volumetric flask and diluted to 50 mL with distilled water. The resulting mixture was titrated with 0.1 N NaOH to a pH value of 8.1 ± 0.2. Total acidity was calculated as percentage of citric acid on a fresh weight basis (Bhat *et al*., 2011). Citric acid equivalent was used in this study as pitaya juice naturally contains high amounts (579 mg/L) of citric acid (Stintzing *et al*., 2003). The pH value of RPJ was measured at ambient temperature (27°C) using a calibrated pH meter (Jenway 3505, UK).

Statistical analysis

The results were expressed as the mean of three replications. Data were analyzed using Minitab V.14 software (Minitab Inc., Pennsylvania). The one-way analysis of variance (ANOVA) and Tukey's multiple range tests were used to generate confidence intervals for the differences between the means ($P \leq 0.05$).

Results and Discussion

Microbiological analysis

Microbiological analysis was conducted on fresh (untreated) and UV treated pitaya juice (Table 1). Total Plate Count for fresh juice was 6.01 $\log_{10}$ CFU/mL and the UV treated juice was 3.58 $\log_{10}$ CFU/mL. There was a significant ($P \leq 0.05$) reduction of 2.43 ± 0.21 $\log_{10}$ CFU/mL in the juice after exposure to UV irradiation. Similarly, significant reduction of yeasts and moulds was observed in the treated juice.

According to USFDA (2001), all juice pasteurisation methods must achieve 5 $\log_{10}$ reductions of pertinent microorganisms. In this study, UV treatment was successful in reducing the microbial counts in pitaya juice for both TPC and YMC but failed to meet the requirement by the FDA to ensure a microbiologically safe product. Therefore an additional step to reduce the microbial count in juice prior to UV treatment is needed. In other words, there is a need for hurdle technology.

Similar observations were made by other researchers who concluded that a 5 log reduction was not achieved in juice and nectar samples treated with UV (Guerrero-Beltrán and Barbosa-Cánovas, 2006; Keyser *et al*., 2008; Bhat *et al*., 2011). If remaining yeasts, moulds and microorganisms are able to survive the pH range of the UV treated juice product, the microorganisms will spoil it predominantly (Guerero-Beltrán and Barbosa-Cánovas, 2005).

Table 1. Microbial load ($\log_{10}$ CFU/mL) in pitaya juice after exposure to ultraviolet (UV) irradiation

Treatment	Total Plate Count	Yeasts and Moulds Counts
Before UV	6.01±0.31[a]	6.20±0.52[a]
After UV	3.58 ± 0.16[b]	3.50 ± 0.34[b]

*Values are the mean of three measurements
[a, b] Different superscript letters on the same column indicate significant differences ($P \leq 0.05$)

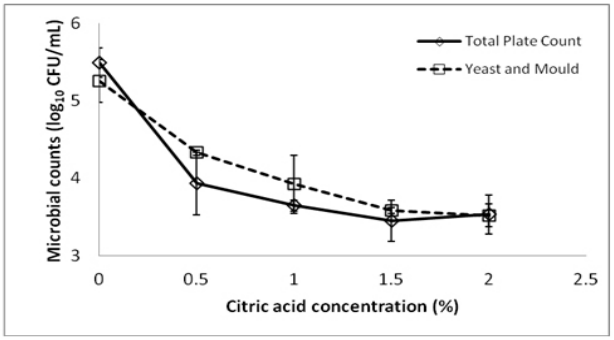

Figure 2. Microbial counts in juice added with various concentrations of citric acid

Effect of citric acid at different concentrations on microbial stability of juice

Citric acid was selected as the antimicrobial agent for this study because it is naturally present in pitaya juice (Stintzing, 2002). Five concentrations of citric acid (0%, 0.5%, 1.0%, 1.5%, and 2.0%) were added into RPJ and the effect on microbial counts was observed (Figure 2). The results indicated that there were significant differences ($P \leq 0.05$) on the TPC and YMC of pitaya juice when citric acid was added. Bacterial growth was more susceptible to citric acid than yeasts and moulds when pitaya juice was added with citric acid. From the reduction of microbial counts, 1.5% and 2.0 % citric acid were found to be the most effective in reducing both bacterial and YMC counts. After addition of 1.5% citric acid, pH value of RPJ became 2.91, which is below the pK_a value (3.14) of citric acid. In other words, citric acid in RPJ was in undissociated form, and this is the form of acid which has antimicrobial activity (Davidson, 2001).

These findings show that addition of 1.5% citric acid into RPJ resulted in reduction of TPC by 2.04 $\log_{10}$ CFU/mL while 2.0% citric acid resulted in 1.96 $\log_{10}$ CFU/mL reduction. For YMC, addition of 1.5% and 2.0% citric acid reduced counts to 1.67 $\log_{10}$ CFU/mL and 1.73 $\log_{10}$ CFU/mL, respectively.

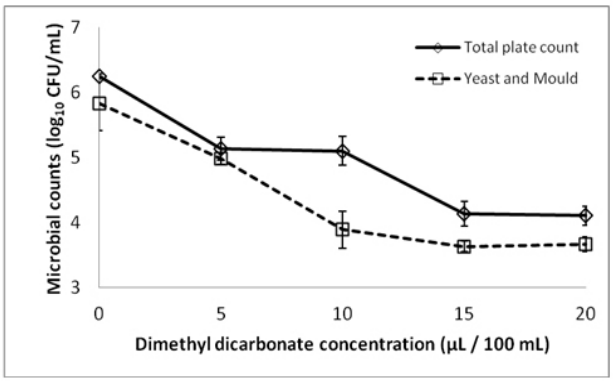

Figure 3. Microbial counts in juice added with various concentrations of dimethyl dicarbonate

Table 2. Comparison of microbial reduction by various treatments

Treatments	Log reduction (log_{10} CFU/ml)	
	Total Plate Count	Yeast and Mould
Ultraviolet (UV)	2.43	2.70
Citric acid (1.5 %)	2.04	1.67
Dimethyl dicarbonate (15 µL/100 mL)	2.11	2.23
Combined treatment	4.12	4.14

Since there was no significant difference ($P > 0.05$) in microbial reduction between the two RPJ juice samples (with 1.5% and 2.0% citric acid) and due to economic reasons, 1.5% citric acid was selected for use in the later part of this study to combine with UV irradiation.

Effect of dimethyl dicarbonate (DMDC) at different concentrations on microbial stability of juice

The effect of adding DMDC into pitaya juice as an antimicrobial agent was tested through five different concentrations of DMDC (0, 5, 10, 15 and 20 µL/100mL). Microbiological analysis was conducted 24 hours after DMDC addition. The result showed that addition of DMDC significantly ($P \leq 0.05$) reduced the microbial counts in RPJ. As illustrated in Figure 3, DMDC concentrations up to 10 µL/100 mL significantly ($P \leq 0.05$) reduced YMC while above this concentration; there was no significant ($P > 0.05$) difference. There were no significant ($P > 0.05$) differences in TPC among the different DMDC concentrations (Figure 3). Addition of 20 µL/100 mL DMDC resulted in the highest reduction of TPC with 2.14 log reductions. Divol *et al*. (2005) reported that 20 µL/100 mL DMDC was very effective against *Zygosaccharomyces bailii*, which is very resistant to most fungicides. The results indicated that among the DMDC-added samples, juice with 5 µL/100 mL DMDC had the highest bacterial count (5.13 log_{10} CFU/mL), and higher concentration of DMDC resulted the higher microbial reduction. However, after a certain point (15 µL/100 mL), the curve showed steady trend (Figure 3).

Microbiological inactivation by combined methods

Non-thermal technologies have been combined to enhance the inactivation of foodborne microorganisms (Ross *et al*., 2003). In this study, comparisons were made between the individual microbial inactivation treatments (UV, citric acid and DMDC) and its combination (Table 2). Through visual observation, no colour changes were visible. The combined treatment was addition of 1.5% citric acid and 15 µL/100 mL of DMDC prior to ultraviolet radiation. Citric acid and DMDC were added prior to UV treatment to overcome the limitation of the UV pasteuriser, i.e. the penetration power. When antimicrobial agents were used, the microbial counts were reduced, thus increasing bactericidal effect of UV light to further reduce microbial counts in the juice.

The results indicated that combined treatment produced a higher microbial reduction than using any one individual method alone, i.e. more than 4 log reduction for both TPC and YMC. This is twice more effective than when individual treatment methods were used. This result indicates that the non-thermal combined treatment of 1.5% CA, 15 µL/100mL DMDC and UV irradiation was successfully applied to reduce the microbial load in RPJ.

Several different combinations of non-thermal technique have been reported to reduce microbial counts in juices. Addition of antimicrobial agents to mango nectar before UV treatment could improve the shelf life at low temperature storage (Guerrero Beltrán and Barbosa-Cánovas, 2006). Noci *et al*. (2008) reported that microbial reduction achieved after UV irradiation was 2.2 log_{10} cycles, while pulsed electric fields led to a reduction of 5.4 log_{10} cycles. However, when the two hurdles were combined, the microbial reduction achieved more than 6 log_{10}.

The use of DMDC in this study was to reduce yeasts and moulds in the juice prior to UV exposure so as to allow a more efficient microbial inactivation by the UV light. Unfortunately, the combined method for microbial reduction did not satisfy the regulations set by United States Food and Drug Administration regulation for juice production (USFDA, 2001).

Conclusion

Ultraviolet irradiation alone was found effective in reducing microbial counts of RPJ to 2 log_{10} reduction. Similarly, addition of citric acid or DMDC into RPJ resulted in 2 log_{10} microbial reduction. Although the combined treatment did not achieve a 5 log_{10}

microbial reduction, it resulted in two-fold microbial reduction compared to individual treatments and this is very promising. Future improvement on increasing the microbial lethality of combined treatments up to 5 $\log_{10}$ of food pathogens is recommended.

Acknowledgments

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