

## Pulsed light treatment as an alternative technology to extend shelf life of fresh-cut Yardlong bean (*Vigna unguiculata*)

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### Abstract

Pulsed light (PL) treatment is a non-thermal method for microbial decontamination on the surfaces of fresh-cut produce. The effect of pulsed light fluencies on microbiological stability and quality changes of fresh-cut yardlong beans were determined. Pulsed light treatments were carried out using an automatic laboratory flash lamp system (Steribeam XeMaticA-2L Kehl, Germany) at different fluencies (1.8 J/cm<sup>2</sup>, 5.4 J/cm<sup>2</sup>, 9.0 J/cm<sup>2</sup> and 12.6 J/cm<sup>2</sup>). Microbiological quality (colour changes and textural changes) of fresh-cut yardlong beans stored at 4±1°C were monitored over 14 days. Results show that, the application of PL treatment at high fluencies allowed extension of microbiological shelf life up to 3-7 days in comparison to untreated samples. Apart from that, PL treated sample has no significant difference on the texture and colour as compared to untreated sample of fresh-cut yardlong bean. As a conclusion, the application of PL at dose 9.0 J/cm<sup>2</sup> has increased the shelf life of fresh-cut yardlong bean while maintaining the quality when stored at 4±1°C.

### Keywords

Ready-to-eat  
Yardlong bean  
Pulsed light  
Non-thermal

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### Introduction

The demand for fresh-cut vegetables has increased rapidly as it offers consumers highly nutritious, convenient and fresh-like qualities (Soliva-Fortuny *et al.*, 2003; Ramos-Villarreal *et al.*, 2011). It is well known that fresh-cut processing which include slicing and/or cutting of fresh vegetables promotes a faster physiological deterioration, biochemical changes and microbial degradation of the products which may result in the degradation of colour, texture and flavour of the vegetables (O'Beirne and Francis, 2003). Apart from that, slicing can cause spreading of the pathogenic microorganism over the cut surfaces which may lead to food borne diseases (Ramos-Villarreal *et al.*, 2011). In order to deal with the increasing demand of ready-to-eat fresh vegetables, the quality of fresh-cut yardlong beans needs to be improved.

In Malaysia, fresh-cut yardlong beans usually consumed raw as 'ulam' accompaniment with plain rice and 'sambal' (chili paste). The 'ulam' are equivalent to salad in other countries. It is a highly nutritious vegetable which act as a source of protein, vitamin A, thiamin, riboflavin, iron, phosphorus, and potassium, Yardlong beans also contain vitamin C, folate, magnesium, and manganese. However, yardlong beans are commonly contaminated with campylobacters thus poses a health risk to consumer (Norzaleha *et al.*, 2003; Chai *et al.*, 2007). Strategies

to minimize the risks consuming fresh-cut vegetables ('ulam') includes electron beam, gamma irradiation, hot water, ozone and vacuum-steam has been studied to enhance the quality and prolong the shelf-life of fresh-cut vegetables (Gonzalez-Aguilar *et al.*, 2010). Pulsed light technology has the potential to be an alternative treatment to extend the shelf life of fresh-cut yardlong beans.

Pulsed Light (PL) technology is a non-thermal emerging treatment for killing pathogenic and spoilage microorganisms in foods, including bacteria, yeasts, molds, and viruses, thus extending their shelf life (Ramos-Villarreal *et al.*, 2012). The treatment consists of applying a series of very short, high power pulses of broad spectrum light. Pulsed light technology has the potential to decontaminate fresh produce. The inactivation depends on the doses (J/cm<sup>2</sup>) and the number of pulses which will occur through several mechanisms including chemical modification and DNA ruptures, protein denaturation and other cellular alteration. Pulsed light has been applied to decontaminate packaging materials such as plastics films and bottles yet becoming the novel food decontamination technology. In this study, pulsed light treatment will be used to reduce surface contamination of fresh-cut yardlong bean.

The application of pulsed light treatment on fresh-cut vegetables is still limited. In the pulsed light processing, treatments fluencies have to be

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optimised for different food products to extend its shelf life while maintaining the quality (Aguiló-Aguayo *et al.*, 2013). Oms-oliu *et al.* (2010) reported that the application of pulsed light at 4.8 J/cm<sup>2</sup> could extend the shelf life of fresh-cut mushrooms without dramatically affecting the textures and antioxidant properties. High fluencies (12 J/cm<sup>2</sup>) were required to reduce 3 log and 2 log growth of *Escherichia coli* and *Listeria innocua* in fresh-cut mushrooms (Ramos-Villaruel *et al.*, 2012). Experiment with carrot slices shows that the application of pulsed light may reduce loads of *Saccharomyces cerevisiae* inoculated on the product by 3 to 4 log cycles (Kaack and Lyager, 2007). There is still no research done to investigate the application of PL treatment on the shelf life of fresh-cut yardlong beans.

The objective of this study is to investigate the effect of different dimension of fresh-cut yardlong beans on microbial inactivation efficiency of pulsed light treatment, this study also aimed to investigate the effect of pulsed light fluencies on microbiological stability and quality changes of fresh-cut yardlong beans.

## Materials and Methods

### Sample collection

Ten kilogram of fresh yardlong bean (*Vigna unguiculata*) were purchased at the Department of Agriculture Selangor, Malaysia, which are sufficient to undertake all the experiments. The fresh yardlong bean were carefully selected to be identical in terms of ripening index stage, shape, size, colour with no blemish or damage.

### Experimental design

Whole yardlong bean including the surfaces and tools in contact with (working area, chopping board and knives) were washed and disinfected. Then, the yardlong bean were rinsed with running tap water, finally dried and cut into three different dimensions which are, Batonnet cut, French cut and thinly sliced. Then, 80 g of each sample were packed in polypropylene (PP) films. Once sealed, the samples were treated by pulsed light for 2 minutes using an automatic laboratory flash lamp system (Steribeam XeMaticA-2L Kehl, Germany). Untreated samples of each type of cuts were prepared as a control. Aerobic count and yeast and mold counts were performed immediately after PL treatments of the cut samples. Three counts were obtained each time of triplicate packages.

The preferred type of cut were selected and treated with four different fluencies (1.8 J/cm<sup>2</sup>, 5.4

J/cm<sup>2</sup>, 9.0 J/cm<sup>2</sup> and 12.6 J/cm<sup>2</sup>). The duration of each pulse was 0.3 ms with a fluence of 0.6 J/cm<sup>2</sup> from two lamp situated 10 cm below and above the sample holder. The fluence was calculated by using the following formula:-

$$\text{Fluence (J/cm}^2\text{)} = \text{Running time (min)} \times \text{Pulses} \times 0.6 \text{ (J/cm}^2\text{)}$$

\*Where 1 min is approximately to 3 pulses

Samples were stored at 4±1°C for 16 days and removed for quality analysis after 0, 4, 8, 12, 14 and 16 days. The analyses included microbiological (total aerobic count and yeast and mould count), texture and colour analysis.

### Microbiological analysis

Aerobic count and yeast and mold counts were performed immediately after PL treatments of the cut samples. Three counts were obtained each time for the triplicate packages. In sterile conditions, 10 g of each sample were homogenized for 2 min with 90 mL of 0.1% sterile peptone water in stomacher bag. Serial dilutions of the homogenates were poured in plate count agar (PCA) at 35±1°C for aerobic bacteria counts and in potato dextrose agar (PDA) at 25±1°C for yeast and mold counts respectively. The PL treated sample results were compared to control for both aerobic counts and yeast and molds counts (AOAC, 1990). All microbiological analyses were carried out in triplicates, and the results were expressed as log colony forming units per gram (log CFU/g). Data shown are the mean of triplicates ± standard deviation.

### Texture measurement

Textures of fresh-cut yardlong beans were evaluated at every 0, 4, 8, 12, 14 and 16 days of storage using texture analyser (TAXT2i, UK). The measurement was taken as the force required for a needle probe with 1.0 g load cell and 5 mm s-1 test speed to penetrate the fresh-cut surface of the yardlong bean held horizontal to the probe. The maximum peak measured during the test was taken as firmness (Rocculi *et al.*, 2009).

### Colour measurement

Colour changes during the storage were evaluated using a Minolta CR-300 chroma meter (Konica Minolta Sensing, Inc., Japan). The instrument was calibrated using a standard white plate. The *b*\* value (yellowness), *a*\* value (redness), *L* value (lightness) were determined for pulsed light treated and untreated samples.

Table 1. Aerobic count and yeast and molds count (expressed as log CFU/g) on fresh-cut yardlong beans treated with pulsed

Sample (dimension)	Untreated	Pulsed Light (4.5 J/cm <sup>2</sup> )
Aerobic count		
Botennet cut	4.45 ± 0.03 <sup>Aa</sup>	3.91 ± 0.05 <sup>Ab</sup>
Thinly sliced	4.28 ± 0.06 <sup>Aa</sup>	4.07 ± 0.08 <sup>Aa</sup>
French cut	4.31 ± 0.06 <sup>Aa</sup>	4.05 ± 0.07 <sup>Aa</sup>
Yeast and molds count		
Botennet cut	4.15 ± 0.08 <sup>Aa</sup>	3.61 ± 0.14 <sup>Ab</sup>
Thinly sliced	4.26 ± 0.05 <sup>Aa</sup>	3.97 ± 0.08 <sup>Aa</sup>
French cut	4.27 ± 0.14 <sup>Aa</sup>	4.00 ± 0.11 <sup>Aa</sup>

\*values design by the same letter are not significantly different ( $p < 0.05$ ). Lower case letter are used for comparison between treated and untreated sample

### Statistical analysis

The data were analysed using one-way ANOVA (Analysis of Variance) followed by Tukey's test at confidence level 95% to determine any significant differences between difference pulsed light fluencies. Differences at  $p < 0.05$  were considered as significant. All statistical analysis was performed using Minitab version 16 (Minitab Inc., Minneapolis, Minn., U.S.A). The experiment was done in triplicates.

### Result and Discussion

#### *Effect of different dimension of fresh-cut yardlong beans on microbial inactivation efficiency of pulsed light treatment*

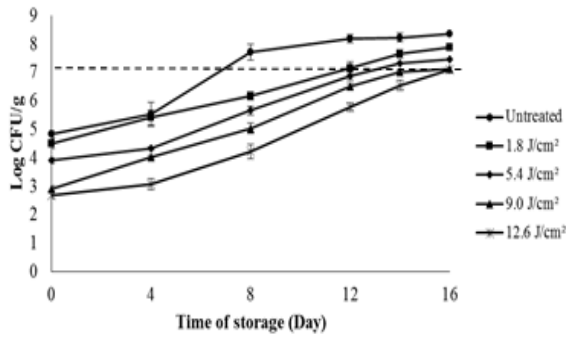
The results of aerobic count as well as yeast and mold count were shown in Table 1. Higher microbial counts were observed for the untreated sample compared to pulsed light treated fresh-cut yardlong bean. Result obtained show that there are no significant effects of pulsed light between each type of fresh-cut yardlong bean. Pulsed light treated on Batonnet cut yardlong bean has significantly lower microbial counts compared to the untreated sample. This shows that pulsed light treatment was more effective on Botennet cut. This is probably because the Botennet cut has smooth and non-reflecting surface accordance to the study reported by Koutchma *et al.* (2010) where the rough surfaces hinder inactivation due to cell hiding while very smooth surfaces, surface reflectivity and cell clumping limit the degree of microbial reduction. It is well known that the surface topography greatly influences the efficacy of pulsed light treatment (Woodling and Moraru, 2005).

#### *Effect of PL fluencies on microbiological stability and shelf life of fresh-cut yardlong beans*

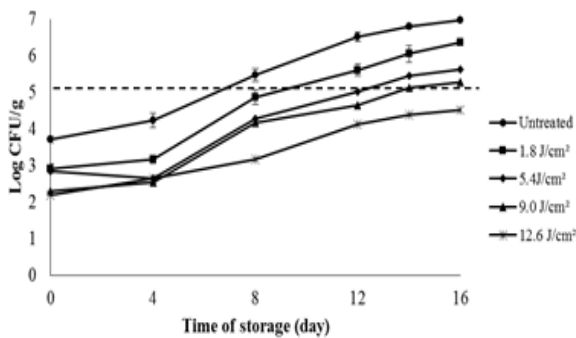
The application of pulsed light technology has significantly ( $p < 0.05$ ) reduced the total plate counts of fresh-cut yardlong bean treated with pulsed light during the storage. Figure 1 show an increasing in aerobic and yeast and mold counts during storage.

However, the counts on pulsed light treated sample were significantly lower than those occurring in the untreated sample. Among four fluencies (1.8 J/cm<sup>2</sup>, 5.4 J/cm<sup>2</sup>, 9.0 J/cm<sup>2</sup> and 12.6 J/cm<sup>2</sup>) of pulsed light, 9.0 J/cm<sup>2</sup> and 12.6 J/cm<sup>2</sup> show higher microbial inactivation in comparison with those treated with 1.8 J/cm<sup>2</sup> and 5.4 J/cm<sup>2</sup>. This indicates that the higher pulsed light fluence, the greater effects on microbial inhibition.

Study by Aguiló-Aguayo *et al.* (2014) on fresh-cut avocado show the same trend where exposure to the highest dose led to the highest reductions in aerobic mesophylic microorganisms and inhibited the proliferation of yeasts and molds for 3 days, prolonging their microbiological shelf life up to 15 days. Similar findings were reported by Oms-Oliu *et al.* (2010) and Ramos-Villaruel *et al.* (2012). Microbial growth on fresh-cut yardlong beans during 16 days of storage is presented in Figure 1. Result obtained shows that untreated sample have reached maximum aerobic count (7 log CFU/g) after 7 days of storage. However, the application of pulsed light increased the shelf life of fresh-cut yardlong beans up to 14 days. Apart from that, the yeast and molds population were prevalent in fresh-cut yardlong beans. According to Jacxsens *et al.* (1999) and Oms-Oliu *et al.* (2010), spoilage in fresh-cut vegetables detected when the yeast counts above 5 log CFU/g. Result shows that samples treated with 9.0 J/cm<sup>2</sup> and 12.6 J/cm<sup>2</sup> maintained yeast and mold counts below 5 log CFU/g up to 14 days. Microbial inactivation by exposure of pulsed light related to ultraviolet (UV-C) part of the spectrum which include photochemical and photothermal effects, which lead to structural changes in DNA of bacteria, viruses and other pathogens, thus preventing cells from replicating (Anderson *et al.*, 2000; Takeshita *et al.*, 2003; Wuytack *et al.*, 2003; Dogu-Baykut *et al.*, 2014). In general, the result shows an increasing in the shelf life of fresh-cut yardlong beans to 3-7 extra days were feasible by using pulsed light at 9.0 J/cm<sup>2</sup>. It can be noted that this fluence is lower than the maximal



(A)



(B)

Figure 1. Aerobic (A) Yeast and mold (B) count on fresh-cut Yardlong bean treated with pulsed light stored at  $4\pm 1^\circ\text{C}$  for 16 days.

limit approved by the FDA which allows a total cumulative treatment of  $12.0 \text{ J/cm}^2$  (FDA, 1998).

### Texture

The texture of pulsed light treated fresh-cut yardlong beans were insignificantly reduced during the storage compared to the untreated fresh-cut yardlong beans. This reduction in texture could be related to an increase in flexibility (loss of crispiness). The loss of cell turgor due to loss in moisture can dehydrate the tissues which lead to increase in elasticity (Martin-Diana *et al.*, 2006).

Result in Figure 2 show that fresh-cut sample treated with low fluencies ( $1.8 \text{ J/cm}^2$ ,  $5.4$  and  $9.0 \text{ J/cm}^2$ ) are better in texture preservation compared to untreated sample. Charles *et al.* (2013) reported that the firmness of fresh-cut mangoes treated by low fluence ( $8 \text{ J/cm}^2$ ) increase could be due to the higher levels of polyamines or the absorption of light and moderate consecutive heating called photothermal effect of pulsed light, inducing the formation of a thin dried film on the surface of the product that could protect against physiological degradation. A substantial decreased was noticed in pulsed light

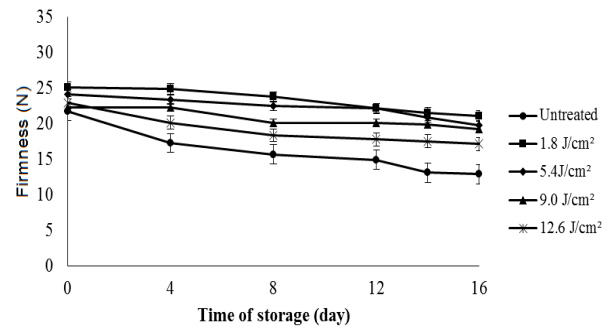


Figure 2. Changes in firmness of fresh-cut yardlong bean treated with pulsed light stored at  $4\pm 1^\circ\text{C}$  for 16 days.

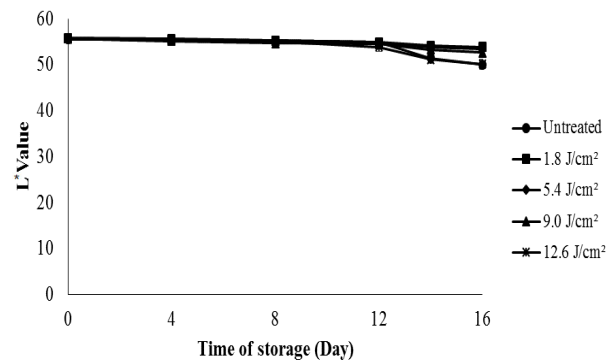


Figure 3. Changes in  $L^*$  value of fresh-cut yardlong bean treated with pulsed light stored at  $4\pm 1^\circ\text{C}$  for 16 days.

treated at high doses ( $12.6 \text{ J/cm}^2$ ) during the storage. Oms-Oliu *et al.* (2010) has also reported the same observation where the use of pulsed light at low fluency ( $4.8 \text{ J/cm}^2$ ) on the texture was first maintained and then slightly increased after 3 days. In the same study, high fluencies, 12 and  $28 \text{ J/cm}^2$ , dramatically affected the textural properties due to thermal damage. Indeed, in spite of the application of short pulses, it has been previously demonstrated that pulsed light increased sample temperature at high fluence can dramatically affects the textural properties of fresh-cut sample due to the thermal damage. Similar trend reported by Gómez *et al.* (2012) where treated fresh-cut apples (at a fluence of  $71.6 \text{ J/cm}^2$ ) softened due to extensive breakage of membranes demonstrated by microscopic observations.

According to the result, it shows that pulsed light at high fluence that have higher microbial inhibition in fresh-cut yardlong beans affected its texture. Thus, combination treatment of pulsed light with texture enhancers such as calcium chloride can be recommended in order to maintain its texture while extending the shelf life.

### Colour

Colour is one of the critical quality properties of fresh-cut product (Oms-Oliu *et al.*, 2010). The  $L^*$  value was selected as the most suitable parameters

to measure the colour changes of fresh-cut yardlong beans. Colour parameter, namely  $L^*$  value of pulsed light treated and untreated fresh-cut yardlong beans are shown in Figure 3 respectively. No significant differences were found for these parameters among different fluencies of pulsed light applied. Decreased in  $L^*$  value was observed in all treatments. Decreased in  $L^*$  value exhibit the sign of browning (Ramos-Villaruel *et al.*, 2014). This might be caused by the physical damage or wounding during preparation which increases respiration and ethylene production, along with associated increases in rates of other biochemical reactions responsible for changes in colour.

Result obtained indicated that the application of pulsed light was not be able to maintain the colour of fresh-cut yardlong beans after 14 days of storage. Therefore, the addition of ascorbic acid will be recommended to maintain the colour parameter during the storage (Gomez-Lopez *et al.*, 2005).

## Conclusion

The application of pulsed light treatment on Batonnet cut yardlong bean has significantly lowered the microbial count compared to the untreated fresh-cut yardlong bean. The fluencies of pulsed light is an important factor in control of microbial growth. This study gives evidence that the higher the pulsed light fluencies, the greater the effect on microbial inactivation and extend the shelf life up to 14 days compared to untreated sample. However, the usage of pulsed light at high dose dramatically affected its final quality as the thermal damage caused dehydration and textural problem on the products. Thus, it is recommended to combine pulsed light treatment with quality enhancers to increase the shelf life while maintaining its quality. These result suggested that the application of pulsed light at 9.0 J/cm<sup>2</sup> is a promising formula for decontamination of fresh-cut yardlong beans although further study are needed to stabilise its colour and texture.

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