Preserving Quality of Fresh Cut Watermelon Cubes for Vending Distribution by Low-Dose Electron Beam Processing

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ABSTRACT

Healthy vending is an untapped market that is expected to grow to meet today's hectic lifestyles. Microbiological safety, quality (shelf-life and sensory attributes), and consumer acceptability are all important factors in determining the success of healthy vending products. The underlying hypothesis was that low dose (~ 1 kGy) electron beam (eBeam) processing by itself, or in conjunction with Modified Atmosphere Packaging (MAP), could be exploited for developing fresh produce-based healthy vending items. The experimental objective was to assess the effect low eBeam dose (~ 1 kGy) with or without MAP, had on bio-burden levels, sensory attributes and consumer acceptability of watermelon cubes. **Electron beam processing had a statistically significant ($P < 0.05$) impact on bacterial (PCA) and fungal (YM) counts on watermelon cubes stored under refrigerated, ambient packaging conditions. The PCA and YM counts on eBeam treated samples did not differ statistically between day 0 and day 7. The PCA counts under MAP storage conditions did not exhibit any difference between day 0 and day 21.** The eBeam treatment did not have a negative effect on firmness or color. eBeam processed watermelon cubes were scored significantly higher ($p < 0.05$) by a consumer taste panel in terms of odor and flavor. The results suggest that low dose (~ 1 kGy) electron beam processing in combination with MAP is a viable approach for developing fresh cut watermelon cube-based vending items.

Key Words: electron beam, eBeam, vending machine, sensory, consumer, bio-burden, watermelon, fruits, healthy vending

1. Introduction

People today lead hectic lifestyles where convenience has become of extreme value. Food items available through vending machines are, however, high in fat, sugar, sodium, and calories. In the United States, more than one-third (78.6 million) of adults are obese and 23 million children are either overweight or obese due to poor dietary habits (*Ogden et al., 2014; Lorson et al., 2009*). The obesity epidemic is responsible for an estimated 300,000 deaths per year and is the second leading cause of preventable death (*Anonymous, 2015*). The health benefits associated with the consumption of fruits and vegetables have been extensively documented and because of the need for convenience and speed, there has been a steady increase in the sales of minimally processed vegetables and packaged fruits (*Ragaert et al., 2004*). Studies show that taste, cost, nutrition, and convenience are some of the main factors that influence consumer's food choices (*Glanz et al., 1998*). Vending machines can potentially be a quick, convenient, and relatively inexpensive approach to make minimally processed vegetables and packaged fruits accessible to people who value convenience. The vending machine market in the U.S. is estimated to be a \$20 billion industry (*Pillai et al., 2013*) and providing packaged fruit and vegetable items for distribution from vending machines located in hospitals, schools, universities, workplaces, and public areas, will seize the untapped opportunity to positively impact nutrition and health on a global scale. Shelf-life, sensory qualities, and consumer acceptability are key factors in determining quality and success of healthy vending products.

Electron beam (eBeam) processing is a non-thermal food processing technology that can be used to extend shelf-life without altering the sensory attributes or consumer acceptance. Electron beam processing is approved by the FDA for use on fresh produce for shelf life extension as long as the dose is ≤ 1 kGy (*FDA, 2014*). The underlying hypothesis is that low dose (< 1 kGy) electron beam processing by itself, or in conjunction with Modified Atmosphere

Packaging (MAP), could be exploited for developing fresh produce-based healthy vending items. Another hypothesis was that consumers' would find eBeam treated watermelon samples as "acceptable". The overall objective was to quantitatively assess the effect that low dose (~ 1 kGy) eBeam processing with or without MAP, had on microbial (bacterial and fungal) bio-burden levels, sensory attributes and consumer acceptability of watermelon cubes. The overall goal of this project was to develop watermelon cubes as a vending machine item.

2. Materials and Methods

2.1. Fruit samples and packaging

Watermelons (*Citrullus lanatus*) with little or no post-harvest processing or disinfectant washing were purchased from a local farmers' market in College Station, TX. The samples were refrigerated (4°C) until they were portioned into smaller 50g samples under aseptic conditions. The 50g samples were placed in polyethylene clamshells (Sambriolo, Santa Maria, CA) for each treatment. For the MAP treatment, the clamshells were then placed in poly nylon vacuum bags (ULINE, Ontario, CA). The bags were flushed multiple times and filled with the appropriate gas mixture (Praxair, Danbury, CT), and then heat-sealed using a commercial sealer (Accu-Seal, San Marcos, CA). The MAP gas mixture used in this study (5% Oxygen, 10% CO₂, 85% Nitrogen) was based on a previous study (Sandhya, 2010). Each of the treatments was prepared in triplicate and each of the experiments was replicated three times.

2.2. Low dose eBeam processing

The packaged samples were transported on blue ice for eBeam processing at the National Center for Electron Beam Research (NCEBR) on the Texas A&M University campus in College Station, TX. The NCEBR is a USDA-APHIS certified facility to treat fruits and vegetables at

low (≤ 1 kGy) doses. A linear accelerator (15kW, 10 MeV) was used for delivering the low eBeam doses from top and bottom. Extensive dose-mapping studies were initially performed to ensure that the measured doses in the fruit samples were ≤ 1 kGy. To achieve the low dose (≤ 1 kGy), the beam energy was reduced by using a defined number of plywood attenuators. The delivered doses were measured using alanine dosimetry and the E-Scan spectrometer (Bruker-BioSpin., Billerica, MA). To ensure accurate dosing with each processing run, “speed checks” were performed on a subset of samples. For each “speed check”, two alanine dosimeters were placed within the watermelon on either end. An X-Acto knife™ was used to create the cavities within the fruit in which the dosimeters (placed in tiny moisture-impermeable, heat-sealed pouches) were placed. The “speed checks” were necessary because it was extremely important to measure the dose within the watermelon rather than just on the top or bottom of the packages. Two such “speed checks” were used prior to each processing run to ensure that the entire fruit received the desired dose. The conveyor speed was adjusted to target the eBeam dose as close to 1.0 kGy as experimentally possible but without exceeding 1.0 kGy. All samples were stored on blue-ice prior to and after eBeam processing.

2.3. Bio-burden Analysis

The bioburden reduction was monitored over a 21-day period. The eBeam processed and non-electron beam processed (control) samples were stored in a refrigerator (4°C) for up to 21 days. The samples were analyzed on days 0, 1, 3, 7, 14, and 21 for Total aerobic Plate Counts (TPC) and Yeasts and Molds (YM) counts. The FDA’s Bacterial Analytical Manual (BAM) protocols were used (*Andrews and Hammack, 2003*) for microbiological enumeration of the samples. Briefly, each 50g sample was aseptically transferred (in biological safety cabinet) into a sterile stomacher bag and 450 ml of Butterfield’s Phosphate Buffer (BPB) (Sigma Chemical CO,

St. Louis, MO). The samples were homogenized for 2 min using a stomacher (Seward 350, London, UK) on the “normal” speed setting. The samples were then serially diluted in BPB and 0.1mL aliquots were spread plated onto Plate Count Agar for enumeration of TPC and Sabouraud’s Dextrose agar (SDA) for YM. All plates (both TPC and SDA) were incubated at 28 °C for 5 days prior to enumeration, and the microbial data was expressed as log CFU/50 g.

2.4. Color Measurements

The surface color of the watermelon samples were measured using a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan). The instrument was calibrated using the white calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan). Color values of L (lightness-darkness), $\pm a$ (red-green) and b (yellow-blue) were measured four times for each sample at each sampling time point and the average values were recorded.

2.5. Texture Analysis

Firmness and textural changes between treatments were measured using the texture analyzer (model TA-XT2i, Texture Technologies Corp., Scarsdale, NY). The analysis was performed on the equatorial region of 10 samples. The texture analysis setup parameters are listed in Table 1. The modulus of deformation (N/mm), force (N), distance (mm) and work to rupture (N.mm) were recorded for all the samples.

2.6. Consumer Acceptability Scoring

The consumer acceptability studies were performed using procedures approved by the Texas A&M University’s IRB procedures (TAMU IRB Protocol # 2012-0463). Thirty-eight untrained volunteers from Texas A&M University participated in the consumer acceptance portion of this study. The samples were prepared by cutting the watermelon into 1-inch cubes, using only the central portion of the watermelon away from the rind. The samples were used in the consumer

acceptance studies no later than 24 hours after eBeam processing. Each treatment was assigned a random 3-digit code to reduce prejudice of sample treatments. Plastic cups were labeled with the random 3-digit numbers in which the samples were placed. Samples were given to the consumers one at a time and unsalted Saltine™ crackers and double deionized water were given between tastings to cleanse their palates. The consumers rated the odor, color, flavor, and texture of each sample using a 9-point hedonic scale from “extremely dislike” to “extremely like”.

2.7. Data Analysis

The original microbial data was converted to log₁₀ CFU/50g to accurately represent the bio-burden of the watermelon samples that were packaged. Data from the replicates were combined and presented showing the mean and standard deviation values (Table 2). The software, GraphPad Prism (GraphPad Software, Inc.,) was used for the statistical analysis.. Statistical analysis of the bioburden data was carried out in two steps. In step 1, the median values (since the microbial data was not assumed to be normally distributed) from the different days of each treatment was compared using the Kruskal-Wallis test of the log transformed data. In step 2, those treatments that showed a statistically significant difference (P<0.05) between the sampling days were then analyzed using the Mann-Whitney test to determine at which day did the bioburden level show statistically significant difference (P<0.05) from day 0. For the consumer acceptability studies, the data was analyzed using the General Linear Model Procedure in Statistical Analysis System (SAS 9.3, 2001). The mean values are reported, and the differences in the mean values were compared by ANOVA and LSD. The differences in the treatments were considered significant when $P \leq 0.05$.

3. Results

3.1. Bio-burden analysis

Table 2 is a compilation of the mean levels of fungal (YM) and bacterial (PCA) bioburden load on the watermelon cubes with and without eBeam treatment when stored for up to 21 days under ambient and MAP conditions. Since these samples harbored natural bioburden levels, there was significant variability in starting numbers with the mean bacterial levels ranging between log 3 and log 5. At the end of 21 days, the mean bioburden levels in the eBeam processed samples ranged between log 6.18 to log 7.64. Statistical tests were performed for the eBeam-processed samples to determine at which time point during storage did the bioburden levels change significantly. Significant differences in bioburden levels (based on Kruskal-Wallis tests) observed in 3 out of the 4 eBeam treated samples namely, PCA and YM counts when stored under ambient conditions. When stored under MAP conditions, only the PCA counts from the eBeam treated samples showed a significant difference. When Mann-Whitney statistical tests were performed, the eBeam-processed, ambient conditions stored watermelon samples did not show statistically significant differences between the sampling days in the eBeam treated samples up to day 7. Under MAP conditions of storage, however, even after 21 days of storage, there was no statistical difference ($P>0.05$) between the untreated (day 0) and the 21 day stored samples.

3.2. Color analysis

Redness (a-values) of watermelon was significantly ($P < 0.05$) higher in eBeam treated (under ambient conditions) immediately after processing on day 0 (Table 3). On day 7, eBeam treatment, by itself or combined with MAP had significantly ($P < 0.05$) redder values when compared to the control samples. After 21 days of storage all treatments were similar in redness compared to the control (Table 3). However, lightness (L-values) did not differ among all different treatment groups throughout the whole storage time ($P>0.05$).

3.3. Texture analysis

On Day 0, eBeam treated watermelon samples (under ambient conditions) were significantly firmer ($P < 0.05$) than the control samples (Table 4). The MAP samples did not have a significant influence on firmness except for samples measured on Day 0, when these watermelon samples were firmer than the control or any other treatment (Table 4). On Day 21, there were no significant ($P > 0.05$) texture differences between the treatments (Table 4). For this analysis, only the flesh of the watermelon was tested due to the fact that the flesh, not the rind, is eaten. Measurements of flesh firmness (N) were relatively close for each sample throughout the storage period, indicating that the eBeam treatment either alone or in combination with MAP did not adversely affect watermelon texture.

3.4. Consumer Acceptability

Table 5 shows the acceptability scores provided by an untrained consumer panel for the different treatments. Thirty-eight volunteer consumers participated and scored the samples based on a 9 point hedonic scale. Consumers rated overall appearance and firmness of watermelon samples equally across all treatments. Watermelons treated with eBeam under ambient conditions yielded a significantly ($p < 0.05$) higher score in the odor attribute (7.42) and was the rated highest in terms of flavor (6.03) (Table 5). The eBeam treated watermelons (under ambient conditions) was highly preferred by consumers in overall appearance (69% scored them 7-9) only second to control samples. All treatments were rated high for this attribute (69% 7-9).

4. Discussion

4.1. Bio-burden

As seen in Table 2, only the eBeam treated samples showed significant differences in the bioburden levels during the 21-day sampling time frame. Three out of the 4 eBeam treatments (PCA-Ambient, YM-Ambient and PCA-MA) showed significant differences between the sampling days implying that the eBeam treatment had an effect on the bioburden levels. None of the control (non eBeam treated) samples showed any significant difference in the counts across the sampling days. Table 2 highlights the differences in bioburden levels (PCA and YM) on the eBeam treated watermelon samples when stored under ambient conditions at different sampling days. Up to day 7, there was no significant ($P>0.05$) difference suggesting that the shelf life was approximately 7 days under ambient storage conditions. Under MAP conditions, the shelf life (based on bio-burden) was increased to 21 days (Table 2). While low dose eBeam processing cannot completely reduce the microbial bio-burden, the potential for it to eliminate pathogens, such as *Salmonella* spp., has been reported previously (Schmidt *et al.*, 2006; Niemira *et al.*, 2013). *Salmonella* spp., a key foodborne pathogen, was the most common pathogen reported in outbreaks related to melons, 38% of which are connected to watermelons (Walsh *et al.*, 2014). The synergistic effects of eBeam processing and MAP on microbial load reduction have been previously studied in fresh fruits and vegetables (Prakash *et al.*, 2000; Han *et al.*, 2004; Palekar *et al.*, 2004). These studies have shown the extension of shelf life without loss of sensory properties or damage to the packaging materials. The bio-burden reduction that can be achieved with eBeam processing could be significant for shelf life. It is estimated that approximately 19 million pounds of fresh fruits are lost annually due to spoilage, of which microbial spoilage plays a major role (Kantor *et al.*, 1997; Brackett, 1994). Barth *et al.* (2009) has reported that loss of shelf-life results in approximately 30-50% shrinkage of fresh-cut fruits. Thus, using eBeam at

low doses even to achieve even a 0.5 -1.0 log reductions in bio-burden could translate to a decrease in the amount of produce lost due to spoilage.

4.2. Color analysis

The application of eBeam alone on Day 0 intensified the redness in fresh cut watermelon samples (**Table 3**). This increase in lycopene content, which is seen when fruits are exposed to stressful environments even at low levels (*Lynch et al., 2009*). Shurong et al. (2006) also found that samples irradiated at doses above 0.5 kGy had a significantly higher red color during days 2 and 4 of storage. The other explanation could be that effect of energy electrons on the plant cell membrane by producing breaks that affect the permeability of the cells (*Hollosy, 2002*) leading to leaching the watermelon pigments on the surface. The effect of 1-5 kGy doses of irradiation on cell membranes and their permeability has been previously shown by Pai & Gaur (1987) and Hayashi et al. (1992). Other studies have also shown that MAP does provide a strategy since it has been widely studied for maintaining the color in fresh produce which is in accordance with our current findings (*Ait-Oubahou, 1999; Shayanfar, 2013; Campbell, 2015*).

4.3. Texture analysis

The texture analysis results (**Table 4**) do not show that eBeam treated samples make the watermelon texture firmer which is in contrast to the previous studies, where it was assumed that irradiation soften the texture (*Yu et al., 1995 & 2006*). This postulated mechanism needs to be verified further. By the last day of the storage there was no difference among the samples ($P>0.05$).

4.4. Consumer Acceptability

Table 5 shows the acceptability scores provided by consumers for all of the various treatments. Consumers rated overall appearance and firmness of watermelon samples equally

across all treatments. The odor and flavor in eBeam processed watermelons were preferred by the panelists. This could be due to the permeation of more aroma as a result of imparting the permeability of watermelon cell membranes. Overall, the use of electron beam processing, alone or in combination with modified atmosphere packaging, did not negatively impact the “acceptability” of the watermelon for the consumers.

5. Conclusion

This study has shown that how even low (~ 1 kGy) eBeam doses are capable of achieving reduced bio-burden and ultimately the quality of fresh watermelon cubes. When used on watermelon cubes, the combination of eBeam processing and MAP can synergistically inhibit microbial growth on watermelon cubes. This can be seen by the extended reduction of microbial growth. Additionally, consumer acceptability and sensory characteristics of the watermelon were not negatively affected by the use of low dose electron beam processing on its own or when used with modified atmosphere packaging. These samples were redder in color and the consumer panelists rated them high in terms of acceptability. Thus, the combination of 1 kGy eBeam with MAP is a suggested approach for preparing watermelon cubes for refrigerated vending machines. The combination of complementary non-thermal technologies such as eBeam and MAP will become a trend in processing fresh produce (*Pillai and Shayanfar, 2015*). Overall, this study shows that the current FDA approved dose of 1 kGy is sufficient to achieve extended shelf-life of a highly perishable product such as watermelon cubes. This can be a practical approach for developing vending items targeted for the healthy vending distribution.

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Table 1. Parameters used in the texture analysis of the watermelon samples

Parameters						Trigger		
Fruit	Pre Test Speed (mm/s)	Test Speed (mm/s)	Post Test Speed (mm/s)	Distance (mm)	Data Acquisition Rate (pps)	Load Cell (kg)	Probe	Force (N)
Watermelon	5	5	5	7	200	25	4mm flat	0.05

Table 2. Mean bioburden levels (bacterial and fungal) (CFU/50g) on watermelon cubes when stored for up to 21 days after being treated with eBeam processing (≤ 1 kGy) under ambient and modified atmosphere (5% oxygen, 10% CO₂, 85% nitrogen) conditions. The detection limit for the bioburden assay was 5000 CFU/50 g = log 3.70. The mean CFU/50 g levels $\pm$ SD are shown.

Storage Period	Ambient conditions				Modified Atmosphere Conditions			
	Plate Count Agar ¹		Sabouraud Dextrose agar ²		Plate Count Agar		Sabouraud Dextrose agar	
	Control	eBeam*	Control	eBeam*	Control	eBeam*	Control	eBeam
Day 0	5.22 $\pm$ 1.42	4.86 $\pm$ 0.76 ^a	4.76 $\pm$ 1.08	3.70 $\pm$ 0.0 ^a	3.70 $\pm$ 0.00	3.70 $\pm$ 0.00 ^a	4.03 $\pm$ 0.58	3.70 $\pm$ 0.0
Day 1	4.70 $\pm$ 0.89	3.70 $\pm$ 0.00 ^a	4.13 $\pm$ 0.75	4.31 $\pm$ 1.07 ^a	4.47 $\pm$ 0.68	4.03 $\pm$ 0.58 ^a	3.70 $\pm$ 0.00	4.03 $\pm$ 0.58
Day 3	4.19 $\pm$ 0.85	4.37 $\pm$ 0.58 ^a	3.70 $\pm$ 0.0	3.70 $\pm$ 0.0 ^a	3.70 $\pm$ 0.00	3.70 $\pm$ 0.0 ^a	3.70 $\pm$ 0.00	3.70 $\pm$ 0.0
Day 7	5.34 $\pm$ 1.62	5.06 $\pm$ 1.57 ^a	5.05 $\pm$ 1.38	4.13 $\pm$ 0.75 ^a	4.85 $\pm$ 1.24	3.70 $\pm$ 0.00 ^a	4.44 $\pm$ 1.29	4.03 $\pm$ 0.58
Day 14	6.92 $\pm$ 2.85	8.81 $\pm$ 0.47	6.46 $\pm$ 2.5	8.78 $\pm$ 0.42	5.97 $\pm$ 1.98	3.70 $\pm$ 0.00 ^a	5.73 $\pm$ 1.79	3.70 $\pm$ 0.00
Day 21	8.03 $\pm$ 3.85	7.44 $\pm$ 3.29	7.99 $\pm$ 3.83	7.43 $\pm$ 3.43	6.46 $\pm$ 2.39	7.64 $\pm$ 1.66 ^a	6.19 $\pm$ 2.16	6.18 $\pm$ 2.19

¹Plate count agar used for enumerating aerobic bacterial bioburden,

²Sabouraud Dextrose agar used for enumerating yeasts and molds,

*Columns with asterisk indicate which eBeam treatment exhibited statistically significant (P<0.05) differences in bioburden levels during the storage period. The bioburden values with the “a” superscript indicate that there was no significant difference in these values within a particular treatment across the storage period compared to day 0.

Table 3. Influence of eBeam processing and MAP storage conditions on watermelon “a” (red-green) and “L” (whiteness-grey) color values

Treatment		Day 0	Day 7	Day 21
Control		21.6 ± 2.4	27.9 ± 3.7	33.9 ± 2.0
eBeam	“a” values	27.9* ± 0.6	36.2* ± 1.2	35.9 ± 1.8
MAP		19.2 ± 2.6	33.3* ± 1.2	35.9 ± 0.4
eBeam + MAP		19.3 ± 1.3	35.1* ± 3.1	32.9 ± 1.5
Control		35.4 ± 1.3	47.7 ± 2.5	48.6 ± 3.9
eBeam	“L” values	36.2 ± 2.2	43.9 ± 3.2	49.4 ± 5.8
MAP		34.1 ± 6.3	46.7 ± 2.6	41.5 ± 3.6
eBeam + MAP		30.2 ± 1.3	45.8 ± 1.2	40.9 ± 0.4

Mean values shown (n=4 sample measurements)

Mean values with * superscript refer to statistically significant differences (P < 0.05)

Table 4. Influence of eBeam processing and MAP on firmness (modulus of deformation (N/mm)) of watermelon samples stored for 21 days

Watermelon		
Treatment	Day 0	Day 21
Air (control)	2.45 ^a ± 0.22	3.52 ^a ± 0.84
eBeam	3.35 ^b ± 0.30	3.82 ^a ± 1.42
MAP	3.84 ^a ± 0.68	3.57 ^a ± 0.61
eBeam + MAP	3.04 ^b ± 0.36	3.28 ^a ± 0.54

Each value is the mean of 10 samples ± SD

Means in columns with different superscripts (eg., a, b) indicate statistically significant differences (P < 0.05)

Table 5. Mean values of consumer scores for watermelon acceptability 24 hours after processing

Treatment	Appearance	Odor	Color	Firmness	Flavor
Air	7.08 ^a ± 1.23	7.42 ^a ± 1.42	7.11 ^a ± 1.37	6.47 ^a ± 1.81	6.03 ^a ± 1.03
eBeam	6.75 ^a ± 1.27	6.61 ^b ± 1.73	6.36 ^b ± 1.13	6.31 ^a ± 1.69	5.14 ^b ± 1.38
MAP	6.94 ^a ± 1.51	6.58 ^b ± 1.46	6.89 ^{ab} ± 1.63	6.69 ^a ± 1.75	5.56 ^{ab} ± 1.50
eBeam + MAP	6.42 ^a ± 2.14	6.31 ^b ± 1.43	6.50 ^{ab} ± 1.72	6.33 ^a ± 1.88	5.22 ^b ± 2.01

Means ± SD in columns with different superscripts (eg., a, b) represent statistically significant differences (P<0.05)