European Journal of Food Research & Review
2(4): 101-110, 2012

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Effects of Irradiation and Chemical Preservatives on the Microbiological Quality of Refrigerated Fresh-Cut Mangoes

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Authors' contributions

This work was carried out in collaboration between all authors. Author EKG designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AAG and JNT managed the analyses of the study and Author VA supervised the research. All authors read and approved the final manuscript.

Research Article

Received 14th June 2012
Accepted 17th October 2012
Published 26th November 2012

ABSTRACT

Fresh-cut mangoes are nutritious and offer consumers freshness, flavour and convenience. They however have a shorter shelf life compared to whole fruits due to their high susceptibility to microbial contamination. The effects of gamma irradiation and chemical preservatives on the microbiological quality of refrigerated fresh-cut mangoes were evaluated. Well matured fruits of Kent and Keitt varieties sliced into cubes were microbiologically analysed initially to determine counts of total viable cells (TVC), coliforms, *Salmonella sp*., *Staphylococcus aureus* and *Escherichia coli*. The samples were subjected to various irradiation doses (0, 1.0, 1.5, 2.0 and 2.5 kGy) and chemical preservatives (sucrose, citric acid, sodium benzoate and a combination of these chemicals in equal proportions) and stored at 6°C and 10ºC for 15 days. TVC was subsequently estimated at 3-day intervals for the treated samples. TVC was estimated as 3.53 ± 0.25 and 4.86 ± 0.38 $\log_{10}$cfu/g for the Kent and Keitt varieties respectively. No coliforms *Salmonella* sp., *E. coli* or *S. aureus* were detected in both varieties. Irradiation at doses of 1.5 kGy to 2.5 kGy in combination with storage at 6ºC was able to eliminate

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all viable cells after 9 days compared to 12 days of storage at 6ºC in the case of chemical preservatives. Irradiation is more effective and ideal compared to chemical preservatives in improving the microbiological quality and therefore extending the shelf life of refrigerated fresh-cut mangoes.

Keywords: Irradiation; fresh-cut mangoes; total viable cells; microbial load, shelf life.

1. INTRODUCTION

Generally, fruits form an important natural staple food and are excellent sources of minerals, vitamins, fibres, anti-oxidants and enzymes. Fresh-cut mangoes are minimally processed fruits and therefore offer consumers freshness, flavour and convenience apart from their nutritional value Lamikanra [1]. The rising public awareness of their health benefits have led to increased production and consumption in recent years Ragaert et al. [2]. Accordingly, sale of fresh-cut fruits such as pawpaw, watermelon and pineapple wrapped in polyethylene has significantly increased in Ghana due to their convenience. Despite the health benefits, fresh-cut fruits have a shorter shelf life compared to whole fruits due to a number of factors. They are highly susceptible to microbial contamination and spoilage, require specific storage conditions and undergo gradual changes in quality Chien et al. [3]. Contamination of fresh-cut fruits with disease-causing microorganisms including human pathogens is common EC [4]. Consequently, there have been increased outbreaks of food poisoning and food infection diseases associated with their consumption, thus posing food safety risks Allong et al. [5], Herndon [6] and UFPA [7].

Several procedures are used to ensure microbiological stability and acceptable hygienic quality of fresh-cut fruits. Production practices such as Good Agriculture Practices (GAP) and Good Hygienic Practices (GHP) in combination with Hazard Analysis and Critical Control Point (HACCP) have been utilized to minimize the level of microbial contamination of fresh-cut fruits. Additionally, appropriate temperature and moisture management, vacuum and modified atmosphere packaging, irradiation and the use of chemical preservatives have been employed to control proliferation of microorganisms in fresh-cut fruits. Irradiation, which serves as a Critical Control Point in HACCP application, has been utilized in controlling microbial contamination of foods over several decades. Studies have consistently shown that irradiation effectively kills bacterial pathogens on fresh and fresh-cut produce Smith and Pillai [8], Niemira and Fan [9]. For several years, a number of chemical compounds such as the chlorine-based preservatives have also been used to reduce bacterial populations on fruits especially before processing or during pre- and post-cutting operations Gómez-López et al. [10] and Gil et al. [11].

In spite of the widespread utilization of irradiation and chemical preservatives for shelf life extension of fresh-cut produce at the international level, there is scanty information on the potential application of these technologies in Ghana. The objective of this study was therefore to investigate the effect/s of gamma irradiation and chemical preservatives on the microbiological quality of fresh-cut mangoes during refrigerated storage.

2. METHODS

2.1 Sample Collection and Preparation

Fifty each of matured Kent and Keitt varieties of mangoes were purchased freshly harvested from farms in Somanya in the Eastern Region of Ghana and transported in plastic crates to the laboratory. The samples were washed in 10% sodium hypo-chlorite solution and rinsed in sterile distilled water. Samples weredivided into three batches for preliminary microbiological quality assessment, irradiation and treatment with chemical preservatives. They were then peeled, seeds removed and processed into cubes.

2.2 Irradiation of Samples

Samples of the fresh-cut mangoes were packaged into polyethylene terephthalate (PET) jars for irradiation at various doses (0, 1.0, 1.5, 2.0, and 2.5 kGy). Irradiation of the samples was carried out in air at a dose rate of 2.42 kGy/h using a Cobalt-60 source at the Gamma Irradiation Facility of the Ghana Atomic Energy Commission. The absorbed dose was determined by using Lithium fluoride photo-fluorescent film dosimeter (SUNNA Dosimeter System, UK).

2.3 Chemical Preservation of Fresh-Cut Mangoes

Samples of fresh-cut Kent and Keitt mangoes were treated with the following preservatives: 30 g/L Sucrose, 3.0 g/L of Citric acid, 2.0 g/L Sodium benzoate and a combination of these three preservatives in equal proportions as the individual chemicals.

2.4 Storage of Chemically Preserved Fresh-Cut Mangoes

The irradiated and chemically-preserved samples and their controls were stored in a refrigerator (IGNIS Model: RWN130) set at 6ºC and 10ºC for 15 days each. A portable laboratory thermometer was kept in the refrigerators to monitor the temperatures throughout the storage period.

2.5 Microbiological Analysis

All the three batches of fresh-cut mangoes were microbiologically analyzed to determine the population of indicator and pathogenic microorganisms. In the case of samples for irradiation and chemical preservation, microbiological analysis was undertaken before and after treatment. Total viable cells, total coliform counts, counts of *Salmonella sp*., *Staphylococcus aureus* and *Escherichia coli* were determined for all the three batches of samples. For each sample, 10 g was weighed into 90 ml Peptone water diluent (0.1% peptone and 0.5 NaCl) and stirred on a mechanical shaker (Junior Obit Shaker, Lab-Line Instrument, USA) for 30 minutes and serially diluted up to 106. One milliliter aliquots from each dilution were dispensed into petri dishes and about 15 ml of the appropriate media was added. Total viable cells were determined on Plate Count Agar (Oxoid, England). Total Coliform counts were determined on Violet Red Bile Agar (Oxiod, England) and *Staphylococcus aureus* was estimated on Baird- Parker (BP) agar (Oxoid, England). *E. coli* was determined on Eosin methylene blue (EMB). All determinations were undertaken in duplicate and three separate experiments were conducted.

All samples were incubated at 37ºC for 24 hours and observed for colonies. Plates that had between 30-300 colonies were selected for the determination of colony forming units per gram (cfu/g) using a colony counter (Staurt Scientific, UK). The number of cfu/g was calculated by multiplying the number of bacteria by the dilution factor.

2.6 Data Analysis

The mean count of total viable cells were calculated and transformed into logarithms. The mean log10(x) values and standard deviations (SD) were calculated on the assumption of a log normal distribution.

3. RESULTS

3.1 Effect of Irradiation on Total Viable Cells of Refrigerated Fresh-Cut Mangoes during Storage

The microbiological quality of fresh-cut mangoes indicated that Kent and Keitt varieties have total viable cells (TVC) of 3.53 ± 0.25 and 4.86 ± 0.38 log10 cfu/g respectively. No coliforms, *E. coli or Staphylococcus aureus* were detected in both varieties. The number of TVC of Kent and Keitt varieties initially increased on the day three for the non-irradiated control sample but decreased over the storage period (Tables 1 and 2). The number of TVC for fresh-cut Kent and Keitt varieties decreased with increasing storage time and increasing irradiation dose at both 6ºC and 10ºC (Tables 1 and 2). No viable cells were detected in the irradiated samples at both temperatures after storage for 12 days and beyond. With regards to samples irradiated at 1.5 kGy to 2.0 kGy, no viable cells were detected after storage for 9 days and beyond at 6ºC and for 12days and beyond at 10ºC. Samples irradiated at 2.5 kGy and stored at 6ºC had no viable cells beyond the first day of storage for the Kent variety and after storage for 9 days and beyond for the Keitt variety.

In the case of samples irradiated at 2.5 kGy and stored at 10ºC, no viable cells were detected after storage for 6 days and beyond for the Kent variety; and after storage for 3 days and beyond for the Keitt variety. The results seem to indicate that whiles there were no appreciable differences in the effect of irradiation on the number of TVC of the two varieties at the storage temperature of 10ºC, irradiation had a greater impact on the TVC of the fresh-cut Kent variety compared to the Keitt variety at a storage temperature of 6ºC (Tables 1 and 2).

3.2 Effect of Chemical Preservatives on Total Viable Cells of Refrigerated Fresh-Cut Mangoes During Storage

There were generally non-uniform but gradual decreases in the number of TVC of the samples of fresh-cut Kent and Keitt varieties treated with chemical preservatives over the storage period of 15 days at 6ºC and 10ºC (Tables 3 and 4). Compared to the untreated control, Citric acid (3 g / L) and Sodium benzoate (2 g/L) reduced the number of TVC of the Kent variety by more than 2 log cycles after 15 days of storage at both temperatures. The mixture of chemical preservatives (Sucrose/Citric acid /Sodium benzoate) eliminated all TVC of both Kent and Keitt samples after 9 days and beyond after storage at 6ºC and 10ºC. With the exception of Sucrose, all the chemical preservatives were more effective in reducing the number of TVC of the Kent variety compared to the Keitt variety.

Table 1. Effect of irradiation on total viable cells of refrigerated fresh-cut Kent mango

Storage temperature	Storage day	Irradiation dose (kGy)				
		0	**1.0**	**1.5**	**2.0**	**2.5**
	0	3.55 ± 0.35	3.43 ± 0.49	3.10 ± 0.99	3.13 ± 0.07	1.20 ± 0.00
	3	4.80 ± 0.00	2.97 ± 3.53	2.55 ± 0.00	2.37 ± 0.00	-
	6	3.60 ± 0.31	2.97 ± 3.53	2.00 ± 0.56	1.50 ± 0.00	-
6ºC	9	3.60 ± 0.03	2.97 ± 3.53	-	-	-
	12	2.95 ± 0.78	-	-	-	-
	15	-	-	-	-	-
	0	3.53 ± 0.25	3.45 ± 0.07	3.43 ± 1.79	3.37 ± 0.58	2.45 ± 0.07
	3	4.93 ± 0.58	3.47 ± 0.84	2.17 ± 0.08	2.25 ± 0.64	1.40 ± 0.00
	6	3.53 ± 0.30	3.20 ± 0.27	2.10 ± 0.69	1.80 ± 0.14	1.10 ± 0.00
10ºC	9	3.45 ± 0.12	2.70 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	-
	12	3.45 ± 0.77	-	-	-	-
	15	3.45 ± 0.00	-	-	-	-

Values are means ±S.D counts of log_{10} cfu/10g; (-) = no colonies observed at minimum detection level of 1.0 log_{10}

Table 2. Effect of irradiation on total viable cells of refrigerated fresh-cut Keitt mango

Storage temperature	Storage day	Irradiation dose (kGy)				
		0	**1.0**	**1.5**	**2.0**	**2.5**
	0	4.70 ± 1.54	4.30 ± 0.38	4.30 ± 1.54	3.50 ± 1.41	3.25 ± 0.05
	3	5.10 ± 0.30	3.60 ± 0.28	3.30 ± 1.08	3.05 ± 1.76	2.90 ± 0.05
	6	3.30 ± 0.55	2.97 ± 1.16	2.80 ± 0.98	2.50 ± 0.70	2.15 ± 0.00
6°C	9	2.30 ± 0.00	2.55 ± 0.00	-	-	-
	12	2.28 ± 0.23	-	-	-	-
	15	2.28 ± 0.12	-	-	-	-
	0	4.83 ± 0.24	4.83 ± 0.81	4.50 ± 0.08	3.17 ± 0.15	2.60 ± 0.79
	3	3.90 ± 0.44	3.83 ± 0.58	2.87 ± 0.71	1.93 ± 0.37	1.33 ± 0.58
	6	3.90 ± 0.82	2.95 ± 0.07	2.37 ± 0.46	1.45 ± 0.64	-
10°C	9	3.33 ± 0.32	2.20 ± 0.00	2.00 ± 0.00	1.30 ± 0.00	-
	12	3.20 ± 0.71	-	-	-	-
	15	3.20 ± 0.11	-	-	-	-

Values are means ±S.D counts of log_{10} cfu/10g;(-) = no colonies observed at minimum detection level of 1.0 log $_{10}$

Table 3. Effect of chemical preservatives on total viable cells of refrigerated fresh-cut Kent mango

Storage temperature	Storage day	Chemical preservatives (g/L)				
		Control	Sucrose (S)	Citric acid (CA)	Na benzoate (SB)	(S/CA/SB)
	0	3.60 ± 0.00	3.86 ± 1.59	3.63 ± 0.00	3.45 ± 0.48	4.50 ± 0.15
	3	3.98 ± 0.28	3.65 ± 0.92	2.83 ± 0.00	3.30 ± 1.69	1.30 ± 0.00
	6	3.85 ± 0.28	3.20 ± 0.28	1.62 ± 0.73	2.26 ± 1.69	1.30 ± 0.00
6°C	9	3.83 ± 0.28	2.20 ± 0.28	1.60 ± 0.70	1.15 ± 0.00	-
	12	3.52 ± 0.28	-	-	-	-
	15	3.20 ± 0.28	-	-	-	-
	0	4.75 ± 0.35	4.50 ± 0.35	4.40 ± 1.55	4.60 ± 0.10	3.50 ± 0.00
	3	3.97 ± 0.51	3.75 ± 1.06	3.60 ± 1.38	3.57 ± 1.61	1.00 ± 0.00
	6	3.97 ± 1.91	3.23 ± 1.01	3.40 ± 0.98	2.95 ± 0.07	1.80 ± 0.00
10°C	9	3.77 ± 1.70	3.23 ± 1.59	3.00 ± 0.00	1.30 ± 0.00	-
	12	3.73 ± 0.04	2.90 ± 1.32	1.90 ± 0.00	1.30 ± 0.00	-
	15	2.65 ± 0.49	2.50 ± 1.20	-	-	-

Values are means ±S.D counts of log_{10} cfu/10g; (-) = no colonies observed at minimum detection level of 1.0 log_{10}; S = 30g/L; CA = 3g/L, SB = 2g/L

Table 4. Effect of chemical preservatives on total viable cells of refrigerated fresh-cut Keitt mango

Storage temperature	Storage day	Chemical preservatives (g/L)				
		Control	Sucrose (S)	Citric acid (CA)	Na benzoate (SB)	(S/CA/SB)
	0	4.90 ± 0.00	4.35 ± 1.62	4.15 ± 0.21	4.69 ± 0.45	4.00 ± 0.00
	3	4.85 ± 0.00	2.82 ± 0.97	3.72 ± 0.30	4.37 ± 1.98	4.02 ± 0.25
	6	3.90 ± 0.00	2.75 ± 2.47	3.00 ± 0.00	3.10 ± 0.00	1.80 ± 0.42
6°C	9	3.20 ± 0.00	2.50 ± 0.00	2.32 ± 0.00	2.00 ± 0.00	-
	12	3.20 ± 0.31	-	-	-	-
	15	3.19 ± 0.05	-	-	-	-
	0	4.53 ± 0.28	4.73 ± 1.35	4.53 ± 1.05	3.59 ± 0.40	3.45 ± 0.35
	3	3.75 ± 0.28	4.13 ± 2.47	4.10 ± 0.65	3.55 ± 0.07	2.90 ± 0.00
	6	3.96 ± 1.29	2.97 ± 1.29	3.40 ± 0.36	3.37 ± 0.23	2.48 ± 0.17
10°C	9	3.90 ± 0.69	2.73 ± 1.50	3.05 ± 0.76	2.70 ± 0.87	-
	12	3.00 ± 0.00	2.50 ± 0.00	3.00 ± 0.00	2.50 ± 0.00	-
	15	2.50 ± 0.00	2.10 ± 0.00	2.90 ± 0.00	-	-

Values are means ±S.D counts of log_{10} cfu/10g;(-) = no colonies observed at minimum detection level of 1.0 log_{10};S = 30g/L; CA = 3g/L; SB = 2g/L

4. DISCUSSION

4.1 Effect of Irradiation on Total Viable Cells of Refrigerated Fresh-Cut Mangoes during Storage

The microbiological quality of fresh-cut mangoes was acceptable largely because they did not contain coliforms and other potential pathogens. Since total viable cells (TVC) represent mainly spoilage microorganisms, their population is significant and therefore largely determines the microbiological quality of fresh-cut mangoes. This finding may be the result of good manufacturing procedures employed in processing the samples as has also been observed elsewhere EC [4].

Irradiation had an impact on microbiological quality of fresh-cut mangoes since the number of TVC reduced over the storage period for both Kent and Keitt at the storage temperatures of 10ºC and 6ºC. The study has shown that the microbiological quality of cut mangoes can be improved by treatment with ionizing radiation at doses less than 2.5kGy. This agrees with Patterson and Stewart [12] and Farkas et al. [13] that irradiation of pre-packaged vegetables and ready to eat meals including fruits at 2.0 kGy and 3.0 kGy reduced the total viable cells to levels below the detection limit of 100 cells/g and counts did not increase significantly during storage at 5ºC. In a related study on apple and pear jam, Mossel [14] and Abadias et al. [15] reported an increase in microbial load within the first 3 days of storage at 10ºC and there after a reduction.Chantanawarangoon [16] reported rapid increases in both the total microbial and yeast and mould counts in the control experiment of mango cubes after 4 days of storage at 5ºC. When storage was extended up to 10 days, there were no significant differences in total microbial and yeast and mold counts of mango cubes stored at 5ºC except the control, which had higher microbial population as was also found in this study.

Temperature seems to contribute to the low microbial counts as seen in Kent and Keitt samples irradiated and stored at 6ºC compared to storage at 10ºC. Several studies have confirmed that good temperature management lowers the rate of physiological activities of fruits and therefore prolongs the shelf life of fruits (Allong et al. [5]; Farkas et al. [13]; Farzana [17]. In a study on non-irradiated fresh-cut ‘Carabao’ mangoes, Izumi et al. [18] noted that storage at 5ºC maintained the quality of mango cubes and extended the shelf-life between 4 to 6 days. Furthermore, Dea et al. [19], experimenting with fresh-cut Kent mangoes found that storage at 12ºC could only extend the shelf-life between 3 to 4 days whiles at 5ºC the shelf life was extended to between 5 to 6 days In this work irradiation has been shown to reduce the number of TVC and therefore the microbial load, thus extending the shelf life of fresh-cut mangoes. The action of radiation is through the radiolysis of water which generates free radicals, which in turn attack the DNA and other organelles in microorganisms, thereby causing their inactivation and eventual elimination Fan et al. [20].

4.2 Effect of Chemical Preservatives on Total Viable Cells of Refrigerated Fresh-Cut Mangoes during Storage

Chemical preservatives are only protective because they are limited to the surface of the fresh cut products. They do not penetrate the tissues of the products and are therefore unable to inactivate the DNA and therefore unable to completely eliminate the total viable cells. Chemical preservatives alter the pH of the medium as well as that of the bacterial membrane resulting in exposure of viable cells to high osmotic concentration Gabrielson [21]. In this study, the chemical preservatives were effective in eliminating the viable cells of

the fresh-cut mangoes especially at the storage temperature of 6^0C after 12 days of storage and beyond. It is important to also note that in the combination preservatives, the chemicals acted synergistically in the mixture to effectively eliminate the viable cells of the fresh-cut mangoes after storage for 9 days and beyond and therefore improved the microbiological quality of the fresh-cut mangoes.

Microorganisms play a very important role in determining the microbiological quality and therefore shelf-life and safety of food products. The impact of chemical preservatives on the microbiological quality of fresh-cut mangoes had been demonstrated by the results of this study. Several chemical compounds have been used to reduce bacterial populations on fruits and they are still the most widely used treatments, either before processing or during pre-cutting and post-cutting operations. Treating fresh-cut mangoes with chemical preservatives can improve shelf-life and microbiological safety by destruction of spoilage microorganisms. In a related study, the shelf life, quality and microbiological safety of minimally processed mangoes were improved by the use of chemical preservatives Ragaert et al. [2] In another study on a closely related fruit, jackfruit (Artocarpusheterophyllus L) Saxena et al. [22] also reported shelf life extension in fresh-cut jackfruit pretreated with Sodium benzoate (0.045% w/v) in combination with proper surface sanitisation.

The results of the present study seem to suggest that temperature contributed to the low microbial counts as seen in Kent and Keitt samples stored at 6ºC as compared to storage at 10ºC. Farzana [17] noted that by reducing storage temperature as was also the case in this study, the rates of metabolism in microbes and fruit tissues can be slowed, thus extending shelf life. It has also been established that good temperature management is very important in prolonging the shelf life of mangoes, and that 2 to 5ºC is the optimum storage temperature range since storage at 0ºC for more than 10 days caused chilling injury Kader [23].

4.3 Comparative Effects of Irradiation and Chemical Preservatives on Microbiological Quality of Refrigerated Fresh-Cut Mangoes during Storage

On the basis of comparing the impact of irradiation and chemical preservatives on microbiological quality of fresh-cut mangoes, the study revealed the effectiveness of irradiation in combination with refrigerated storage in reducing the population of spoilage microorganisms i.e. total viable cells. This study has shown that irradiation doses of 1.5kGy to 2.5kGy in combination with storage at 6ºC were able to eliminate all viable cells after 9 days compared to 12 days of storage in the case of chemical preservatives. The role of irradiation in eliminating microorganisms from food is well documented Smith and Pillai [8], Niemira and Fan [9]. The radiolysis of water by ionizing radiation generates free radicals, which in turn, destroy the DNA and other organelles in microorganisms and thereby inactivating and eliminating them J Am Diet Assoc. [24] and Fan et al. [20]. The capacity of irradiation to eliminate microorganisms in foods without accumulation of residues makes the technology suitable as a food processing tool. Chemical preservatives have been identified as incapable of completely eliminating microorganisms on fresh produce, but rather act as osmotic agents which change the membrane potentials of the microorganisms through enzymatic reactions thereby inactivating them Abadias et al. [15]. Aside not being as effective as irradiation in eliminating viable cells from the fresh-cut mangoes, chemical preservatives have also been known to leave residues in foods, thus creating food safety concerns for consumers.

This study has revealed irradiation as more effective and ideal compared to the use of chemical preservatives in improving the microbiological quality and therefore extending the

shelf life of refrigerated fresh-cut mango.

Subsequent studies will explore the impact of irradiation doses of 1.5 to 2.5 kGy on the sensory qualities of refrigerated fresh-cut mangoes.

ACKNOWLEDGEMENTS

I wish to acknowledge Dr F.K Saalia, Department of Nutrition and Food Science, University of Ghana. Ms Theodosia Adom, Radiological and Medical Sciences Research Institute Ghana Atomic Energy Commission for their encouragement during this research

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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