BENCHMARKING THE MINIMUM ELECTRON BEAM (EBEAM) DOSE REQUIRED TO ACHIEVE STERILITY OF SPACE FOODS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Benchmarking the Minimum Electron Beam (eBeam) Dose Required to Achieve Sterility of Space Foods

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The safety, nutrition, acceptability, and shelf life of space foods are of paramount importance to NASA, especially on long-duration missions. Since food and mealtimes play a key role in reducing stress and boredom of prolonged missions, the acceptability of food in terms of appearance, flavor, texture and aroma can have significant psychological ramifications on astronaut performance. The FDA, which oversees space foods, currently requires a minimum dose of 44 kGy for irradiated space foods. The underlying hypothesis is that commercial sterility of space foods could be achieved at significantly lower doses. Lowering the minimum dose can positively impact the visual appearance, sensory attributes, nutrient content, and overall acceptability of space foods. The focus of this project was to use beef fajitas (an example NASA space food) and employ eBeam processing to benchmark the minimum eBeam dose required for sterility. A 15 kGy dose was able to achieve an approximately 10 log reduction in STEC bacteria, and 5 log reduction in *Clostridium sporogenes* spores. Furthermore, accelerated shelf life testing (ASLT) to determine sensory and quality characteristics under various conditions was conducted. Using GC/MS-olfactory analysis, numerous volatiles were shown to be dependent on the dose applied to the product. Furthermore, concentrations of off –flavor aroma compounds

such as dimethyl sulfide were decreased at the reduced 15 kGy dose. The long-term goal of this project is to collect empirical data to enable NASA to petition the FDA to lower the minimum dose from 44 kGy to significantly lower doses.

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SECTION I

INTRODUCTION

Space food program

History

The nature of foods consumed in space has evolved drastically since astronauts first started eating in space during the Mercury missions (1958-1963). The first food products consumed in flight were bite-sized cubes, freeze-dried powders, and semi-liquids in aluminum tubes (NASA, 2002). As the missions increased in length during the Gemini (1961-1966) and Apollo missions (1969-1972), more emphasis was placed not only on the food and packaging specifications to ensure maximum safety, but also the sensory and nutritional aspects of the food. Thermostabilized items in flexible retortable pouches and irradiated products were available for the first time in 1968 on Apollo 8 (Perchonok and Bourland, 2002). Since then, one of the main focuses of the space food program has been to extend the shelf life of the food.

Currently, there are seven different categories of food consumed on the International Space Station: Thermostabilized, irradiated, rehydratable, natural form, fresh food, extended shelf life bread products, and beverages (Cooper and Perchonok, 2011). Thermostabilized foods are the most common and are heated to a temperature that renders the product commercially sterile, free of pathogens, spoilage microorganisms, and enzymatic activity. Irradiation consists of using ionizing radiation to commercially sterilize meat products, while rehydratable foods are freeze-dried products which are then rehydrated during the mission using either ambient or hot water.

Natural form foods are either low moisture (nuts) or intermediate moisture (dried fruit) commercially available shelf stable products, while fresh foods are typically fruits and vegetables and are only available sporadically. Extended shelf life bread products are products such as tortillas which are specially formulated and packaged to have an 18 month shelf life. The beverages available to astronauts are either rehydratable mixes or flavored drinks (Catauro and Perchonok, 2012; Cooper and Perchonok, 2011). Meals are combinations of these forms of food, with an example of a meal being shrimp cocktail (rehydratable), beef steak (irradiated), corn (rehydratable), baked beans (rehydratable), macadamia nuts (natural form), chocolate pudding cake (thermostabilized), and apple cider (beverage) (NASA, 2009).

Requirements

The National Aeronautics and Space Administration (NASA) has been tasked with developing the technologies to explore a variety of destinations beyond the low Earth orbit. These include near-Earth asteroids, the Moon, and eventually Mars (NASA, 2015). Currently, a number of technologies are being tested and deployed on the International Space Station to achieve NASA's manned space program goals. These technologies include everything from electron beam processing and 3D printing, to resource recycling, habitation systems, nuclear fission, and laser communication devices (NASA, 2013; NASA 2015).

Since food and mealtimes play an instrumental role in space travel by reducing the stress and boredom of prolonged missions, the acceptability of food in terms of appearance, flavor, texture, and aroma can have significant psychological ramifications on astronaut performance

(Perchonok and Douglas, 2009). While retorted products have generally had the highest acceptability and the greatest potential to maintain acceptability for extended periods of time (3-5 years), the amount of energy needed to render the product free of pathogens, spoilage microorganisms, and enzyme activity can also lead to product deterioration, especially after 3 years (Catauro and Perchonok, 2012). Thus, what are needed are validated non-thermal technologies that can guarantee sterility and acceptability in terms of appearance, flavor, texture, and aroma.

Electron beam processing

Ionizing radiation functions by transferring energy to materials by ejecting atomic electrons. These electrons then ionize other atoms in a series of chain reactions. The FDA has approved three main forms of ionizing radiation: gamma, x-ray, and electron beam irradiation. Gamma irradiation is produced when radioactive isotopes emit high-energy photons. While gamma irradiation has a relatively deep penetrating ability, the main ionizing source degrades by about 12.35% annually due to having a half-life of 5.26 years. X-rays are emitted when energetic electrons strike any material, but efficiency rates have been historically inefficient (Cleland, 2007).

In electron beam (eBeam) irradiation, a particle accelerator such as a linear accelerator is used to generate electron beams. There is no radioactive source, and the source is switched on and off as needed. This form of food irradiation has a more shallow penetrating ability, but this is overcome

by either dual exposure or increasing the beam energy parameters up to 10 MeV, the highest amount allowed by the FDA (Miller, 2005).

Electron beam (eBeam) processing is one technology that addresses NASA's needs for technological, financial, and environmental stability. The advantages of eBeam technology include it being non-thermal, a "green technology" (chemical free), having a reduced carbon footprint, and being significantly more cost-effective than other methods of food irradiation (Pillai,2014; Pillai and Shayanfar, 2015). This technology is a proven commercialized technology in the highly regulated medical device and pharmaceutical industries as well as in the commercial food industry.

Since eBeam processing is a non-thermal technology, little or no change should be noticed in the nutritional or sensory attributes of foods treated with eBeam (Lopez et. al., 2000). However, it is critically important that the dose chosen for eBeam processing be optimized (Miller, 2005). The FDA currently requires a minimum dose of 44 kGy for any frozen, packaged meats used as space foods (FDA, 2015). We hypothesize that 44 kGy is unnecessary and the same level of sterility can be achieved at a dose between 8 kGy and 15 kGy (Bhatia and Pillai, 2015).

Shelf life

While it is generally understood that the quality of most food deteriorates over time, quantifying this deterioration can be difficult. Food manufacturers bear the responsibility of determining the maximum time period in which not only does a product remain microbiologically safe, but also

the maximum time period in which the product maintains its quality. For most foods, there is usually a finite period in which the product is considered acceptable for consumption. The time between production and unacceptability is considered a food's shelf life. While products with a relatively short expected shelf life of 6 months or less are easily kept to measure the actual shelf life, determining the long-term sustainability of foods with extended shelf lives can be much more difficult.

Accelerated Shelf Life Study (ASLT)

Accelerated shelf life testing (ASLT), is most often used to test products with an expected shelf life of over one year. Especially in the situation of space foods, where the expected shelf life is 5-7 years, ASLT can be used to estimate the quality of the food throughout its shelf life, without having to keep and test the food for 5-7 years, a practice that would impractical. ASLT follows the basic principles of chemical kinetics, which are used to quantify the effects various extrinsic factors (temperature, humidity, light) have on the rate of deteriorative chemical reactions (Brody and Lord, 2000).

Beef flavor

While the five basic tastes (salty, sweet, bitter, sour, and umami) make up an important part of beef flavor, even the most basic flavors rely heavily on volatile aromas in order to be detected by the consumer (Kerth and Miller, 2015). The aspects of meat quality that affect consumer acceptance the most are taste, texture, and juiciness (Ritota et. al., 2012). With food irradiation, after safety, the taste or flavor of the food is of most concern. While beef flavor is an extremely

complex quality trait, the basic flavor components can be divided into two distinct categories: lipid-derived products and Maillard reaction products (MRP) (Kerth, 2016; Mottram, 1998). Lipid derived products are a result of lipid degradation and oxidation into aromatic compounds, while MRP's are the result of heating amino acids and reducing sugars at high temperatures. Lipid derived products are more common at conventional cooking temperatures, while MRP's tend to only be present when meats are cooked at extremely high temperatures (Kerth and Miller, 2015).

With irradiated beef, not only do the overall number of volatiles increase with eBeam, many volatiles compounds have been shown to increase with dose, specifically aliphatic hydrocarbons such as palmitic acid, stearic acid, oleic acid, linoleic acid, 1,7 – hexadecadiene, and 8-heptadece (Mottram, 1998; Kim et. al., 2004; Kwon et. al., 2008; Li et. al., 2010). These hydrocarbons have been detected at doses as low as 0.5 kGy (Kim et. al, 2004). In beef, potential spoilage indicators are 2-pentanone, 2-nonone, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl hexanoate, ethyl propanoate, ethyl lactate, ethyl acetate, ethanol, 2-heptanone, 3-octanone, diacetyl, and acetoin (Argyri et. al., 2015). These compounds have been described with descriptors such as as sweet , fruity, banana, cheesy, green, fruity, dairy, buttery, and medicinal (Kerth and Miller, 2015).

Experimental objectives

The project was divided into two phases. Phase 1 involved determining the minimum eBeam dose needed to achieve commercial sterility of beef fajitas samples. This was accomplished by inoculating beef fajita samples with defined titers of different microorganisms and bacterial

spores and processing the samples at defined eBeam doses. Phase 2 consisted of an accelerated shelf life study that examined how the nutrient quality and sensory attributes of eBeam-treated beef fajitas changed over time. The goal of the shelf life study was to simulate 5 years of storage at room temperature.

SECTION II

METHODS

Model space food

Beef fajitas (a Mexican meat-based dish) was chosen as a model space food. This dish was chosen based on discussions with NASA food scientists and also because beef fajitas are currently consumed by US astronauts on board the International Space Station. Since the current FDA regulations on minimum dose requirements for space food relate to beef specifically, a beef product was chosen to be the model food for this project.

When prepared at the space food facility, the beef fajitas were grilled before being combined with rehydrated peppers, onions, and sauce. For logistical and convenience reasons, for these experiments, beef fajitas composed of beef, vegetables, and onions were obtained from a retail restaurant. Food (120-140 g per pouch) was placed in retort pouches (Ampac, Hanover Park, IL) and the filled retort pouches were left unsealed and refrigerated at 4 ºC until the subsequent steps of eBeam processing or microbial inoculation.

The retort pouches used in this experiment were composed of a laminate of alternating layers of metal foils and flexible plastics, specifically, Polyester, Aluminum foil, Biaxially oriented Linear Tear Nylon, and Cast Polypropylene (Figure 1). These packages are used for space food due to their decreased weight and low stowage volume (Perchonok, 2009).

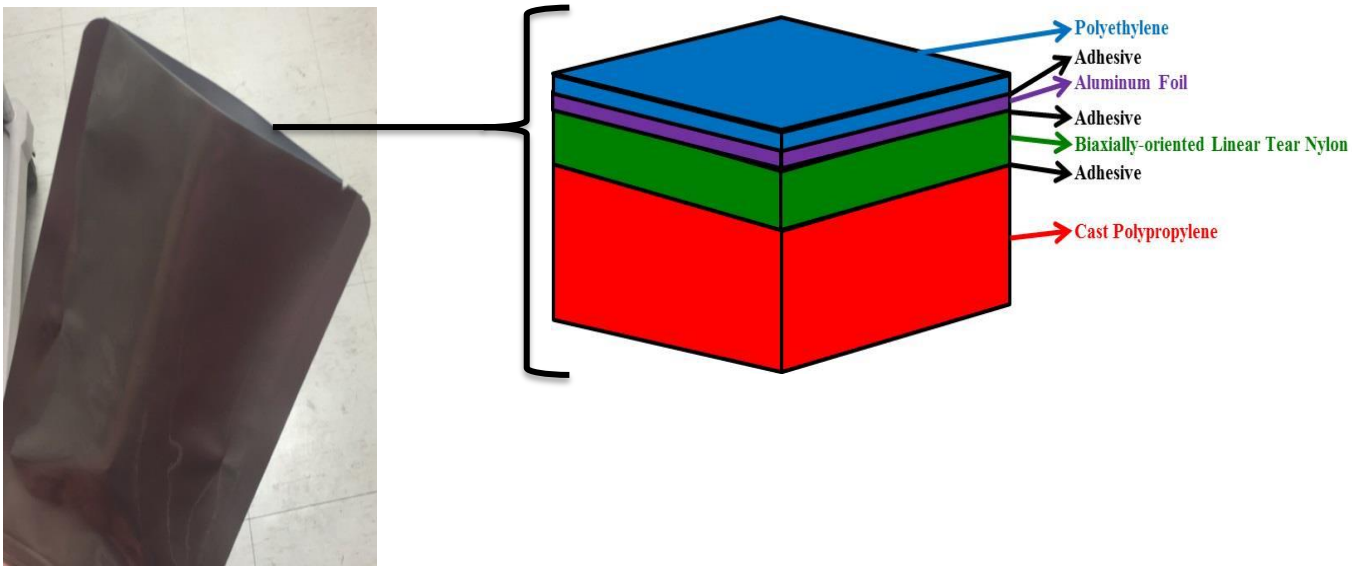


Figure 1. Retort pouch with layer breakdown

Experimental design

Based on discussions with NASA food scientists the model space food that was chosen was beef fajitas. In order to benchmark the minimum eBeam dose required to sterilize space food, an example space food was inoculated with various bacteria that are likely to be found in beef fajitas. Once the products were exposed to eBeam, the remaining microbial population was enumerated on selective media.

Bacterial preparation

Shiga toxin producing Escherichia coli

A cocktail of *E.coli* O157:H7 and non-O157 Shiga-toxin producing *E. coli* (STEC) was prepared using nine bacterial strains. These pathogenic strains were obtained from the USDA-ARS

(USDA-FFSRU, College Station, Texas). Six non-O157 STEC (O26, O45, O103, O111, O121, and O145) and three O157:H7 STEC (ATCC 933, ATCC 8264, and ATCC 43895) were grown on Trypticase Soy Agar (TSA) plates at 37 ºC for 24 hours. Prior to each experiment, an overnight culture of each strain was prepared by transferring a single colony to Trypic Soy Broth (TSB) and incubated at 37 ºC overnight. The overnight cultures were "washed" with Phosphate-Buffered Saline (PBS). The washing protocol was as follows: bacteria were first centrifuged to form a bacteria pellet. The supernatant was poured off and replaced with fresh, sterile PBS. This solution was vortexed before being centrifuged again. The supernatant was once again poured off and replaced with fresh sterile PBS. The tube was then vortexed before another round of centrifugation. This was repeated for a total of three times. Centrifugation was at 4000g for 10 minutes each time (Barizuddin et. al., 2015; Hsu et. al., 2015). After the last washing, the bacteria were suspended in PBS. The optical density (OD-600nm) of each culture was used to determine the approximate concentration of bacterial cells in each suspension and each culture was standardized to 10^8 CFU/ml. A cocktail with a titer of 10^8 CFU/ml was then prepared, containing equal amounts of each strain.

Clostridium sporogenes spores

The method of spore preparation and purification was adapted from Yang et. al (2009). *Clostridium sporogenes* was plated on Reinforced Clostridial Agar (RCA) and incubated anaerobically at 37ºC for 72 hours. A single colony was then transferred into a falcon tube containing reinforced clostridial medium (RCM) and incubated anaerobically at 37 ºC for 24 hours. For this broth culture, Oxyrase for Broth (Oxyrase, Mansfield, OH) was used to maintain

anaerobic conditions. For sporulation, 400 µl of this growth culture was transferred to a falcon tube containing 40 ml of sporulation media (3% tryptone, 1% peptone, 1% ammonium sulfate). To increase the ultimate yield of spores, many tubes were prepared. These cells were heat shocked in a water bath at 80 ºC for 15 minutes. Once cool, the samples were incubated anaerobically at 30°C for 7 days, while shaking at 180 rev/min. After 7 days, microscopy was used to confirm sporulation (Yang et. al., 2009).

For spore purification, the spores were centrifuged at 4,000g for 15 minutes at 4 ºC after which the supernatant was discarded. The pellet was suspended in 40 ml of sterile deionized water and centrifuged again at 4000g for 15 minutes at 4 ºC. The supernatant was discarded and the pellet was re-suspended in 40 ml of PBS containing 500 µg/ml lysozyme. The samples were vortexed for 5 minutes and incubated for 2 hours in a 37 ºC water bath to allow the lysozyme to digest any vegetative cells. The spores were then washed 8 times with 40 ml of sterile ddH_2O via centrifugation (Yang et. al., 2009). The multiple tubes were combined and the purity and concentration of the spore suspension was confirmed via microscopy using a Petroff-Hausser bacterial counting chamber (Hausser Scientific, Horsham, PA). Spores were stored at 4 ºC until processing.

Inoculation

Prior to eBeam processing, 5 grams of beef fajitas (approximately 2.5 grams vegetables and 2.5 grams of beef) was removed from each of the unsealed pouches, cut into very small pieces with a sterile scalpel, and placed in a sterile WhirlPak bag (Whirl-Pak, NASCO, Fort Atkinson, WI).

Before being sealed, this pouch was inoculated with a 1 ml aliquot of the STEC cocktail (approximately 10^9 CFU/g) or *C. sporogenes* spores (approximately 10^9 Spores/g). This bag was then triple bagged in WhirlPak bags, heat sealed, and placed back within its respective retort pouch within the food. This was done so that dose received by the inoculated beef fajitas would be most similar to the dose that would be received in a regular package that is not uniform in thickness. Air was excluded from the retort pouches by gently 'pressing out" the air within the retort pouch before being heat sealed using a heat sealer (Uline, Pleasant Prairie, WI) and placed in a specimen transport bag. The samples were transported on ice to the commercial eBeam facility on campus.

Dose response of C. sporogenes spores in beef fajitas

The objective of this experiment was to determine the dose response of the bacteria spores in the beef fajitas matrix. The same process described above for inoculation was followed for the preparation of samples for this experiment, except instead of placing the inoculated triple bagged 5 g pouches into a larger retort pouch, the pouches were left as is. Samples were processed at target doses of 0, 2, 4, and 8 kGy. Triplicate samples were used.

Electron beam processing

eBeam processing

Electron beam (eBeam) processing was performed at the National Center for Electron Beam Research at Texas A&M University in College Station, Texas. A 10 MeV, 15 kW, linear accelerator was used. Retort pouches were arranged in a flat, single layer. Un-irradiated and un-

inoculated samples were also kept in order to analyze the starting background microbial population of the food. Triplicate samples were used.

Dosimetry

To measure the absorbed dose, alanine (L-α-alanine) dosimeters (Harwell Dosimeters, Oxfordshire, UK) were placed below the retort pouch so that any dose received by the dosimeter was most likely to indicate the actual dose exposed to the inoculated product. A Bruker E-scan spectrometer (Bruker, Billerica, MA) was used to measure the delivered eBeam dose (Praveen et al., 2013).

Microbial enumeration

After eBeam processing, the samples were refrigerated at 4 ºC until microbial analysis. The inoculated pouch was removed from the retort pouch in a biological safety cabinet. Since the inoculated food was triple bagged, the outer two bags were removed, and the innermost pouch containing the actual inoculated food was cut open. The sample inside was considered the (undiluted) 10^0 dilution. The 5 grams of beef fajita and the pouch were then fully immersed within a sterile 100 ml glass bottle containing 45 ml of Butterfield's Phosphate Buffer (BPB). This was considered the 10^{-1} dilution. Subsequent ten-fold serial dilutions were made with BPB by transferring 100 µl into 900 µl dilution blanks. All instruments, such as scissors and spatulas used during this process were pre-sterilized in an autoclave or flame sterilized. The surviving organisms were enumerated on selective media as follows: For the samples inoculated with *E. coli*, modified mTEC agar was used. To enumerate non-*E. coli* bacteria present in the food,

samples were also plated on TSA. These plates were incubated at 37 ºC for 48 hours. Samples inoculated with *C. sporogenes* spores were plated on RCA and incubated anaerobically for 72 hours.

Table 1. Organism, media, and incubation conditions.

Target Organism	Media Used	Incubation Conditions
E. coli	Modified mTEC TSA	Aerobically at 37 ºC for 48 hours
C. sporogenes spores	RCA TSA	Anaerobically at 37 ºC for 72 hours

Accelerated shelf life testing (ASLT)

Retort pouches (triplicate samples) were filled with 100 g of commercially purchased beef fajitas and sealed using a vertical vacuum chamber (Audion Elektro BV, Weesp, Netherlands) allowing a headspace of 10 cm^3 prior to being irradiated at 0, 15, and 44 kGy. Irradiated samples were allowed to equilibrate at room temperature for approximately 24 before being placed at 4°C, 25°C (room temperature), and 55°C for 1 month. GC/MS analysis, bacterial enumeration, and pH were measured at the beginning (time = 0) and end (time = 1 month) of the experiment. Triplicate samples were used. The overall experimental design is shown in Table 2.

Table 2. Experimental design of the shelf life study

Dose (kGy)	Immediately after eBeam	After 1 month		
		4 °C	25 °C	55°C
0	A, B, C	-	-	-
15	A, B, C	A, B, C	A, B, C	A, B, C
44	A, B, C	A, B, C	A, B, C	A, B, C

GC/MS olfactory

Gas Chromotography/Mass Spectoscopy – Olfactory (GC/MS Olfactory) was used in order to analyze the volatile compounds of the samples. Samples that were not analyzed immediately were rapidly frozen using liquid nitrogen and stored at -80°C until sampling. Approximately one hundred grams of each beef fajita sample was placed in a 473 ml glass jar with a Teflon lid and heated to 60 °C in a water bath for 20 minutes to mimic the temperature at which the products would normally be consumed. A solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 μm Carboxen/polydimethylsiloxane [PDMS], Sigma-Aldrich, St. Louis, MO) was then inserted through the lid and headspace volatiles were collected for 2 hours.

After collection, the SPME was removed from the jar and inserted into the injection port of a gas chromatograph (GC; Agilent Technologies 7920 GC, Santa Clara, CA) where it was desorbed at 280°C for 3 minutes . The sample was then loaded onto a multidimensional gas chromatograph and into the first column (30 m x 0.53 mm ID/BPX5 [5% phenyl polysilphenylene-siloxane] x 0.5 μm, SGE Analytical Sciences, Austin, TX). The temperature increased from 40°C to 260 °C at a rate 7 °C per minute. After the second column (30 m × 0.53 mm ID [BP20 - polyethylene glycol] × 0.50 μm, SGE Analytical Sciences, Austin, TX), the column was split three ways: one valve went to the mass spectrometer (Agilent Technologies, 5975 series MSD, Santa Clara, CA) while the other two went to two separate sniff ports, which were heated to 115°C and fitted with nose pieces. The sniff ports and accompanying software for analyzing volatile aroma are a part of the AromaTrax program (MicroAnalytics-Aromatrax, Round Rock, TX).

Bacterial enumeration

Aerobic bacteria were enumerated from the samples in the same methods as described before. Liquid from the beef fajitas pouch was considered the 10^0 sample, while 5 g beef fajitas stomached in 45 ml BPB was considered the 10^{-1} dilution. Subsequent ten-fold serial dilutions were prepared from the stomached mixture. Ultimately, $10^0 – 10^{-2}$ were plated on TSA. Samples were plated prior to irradiation in order to determine the starting bacterial population of the un-irradiated samples, 24 hours after irradiation to ensure starting sterility, and once again at the end of the shelf life study.

pH analysis

Five grams of each product was homogenized with 45 ml of sterile double deionized water for 60 seconds using a bench top stomacher (Stomacher 400 Circulator, Seward, West Sussex, UK). The pH of the homogenized samples was measured using a calibrated pH probe (calibrated with stock solutions of pH 4 and pH 7) (Corning, 430 pH meter, Corning, NY).

Shelf life

The quality and subsequent deterioration of a food is not determined by one simple chemical reaction, rather, numerous reactions occur simultaneously to produce a detectable change in the product. It can still be assumed that some reaction happened to cause the change. This can be simply expressed using the following schematic:

Reagents → *Products*

Furthermore, assuming that the loss of one reagent is equal to the gain of one product, the rate of change can be expressed as:

$$-\frac{dR}{dt} = \frac{dP}{dt}$$

Equation 1. General reaction rate

With volatile compounds having a profound effect on the aroma and taste of a food, and with pH having an equally large effect on the taste and stability of a food, they serve as an aggregate measure of these deteriorative reactions.

Statistical analysis

Data was analyzed using Student's t-test to evaluate the difference between treatments. For the GC/MS-olfactory data, least squared means were calculated and when significance was detected in the least squared means table, differences were analyzed using a Student's t- test with an alpha value of 0.05. This was done using JMP Software (JMP®, Version 9.0.0, SAS Institute Inc., Cary, NC, 1989–2010).

SECTION III

RESULTS AND DISCUSSION

Achieving sterility

The starting concentration of background aerobic bacteria in the commercially purchased beef fajitas samples was determined to be 5.59 ± 1.16 CFU/25 g (Table 3). The background bacterial populations were comprised of both Gram negative and Gram positive rod shaped bacteria.

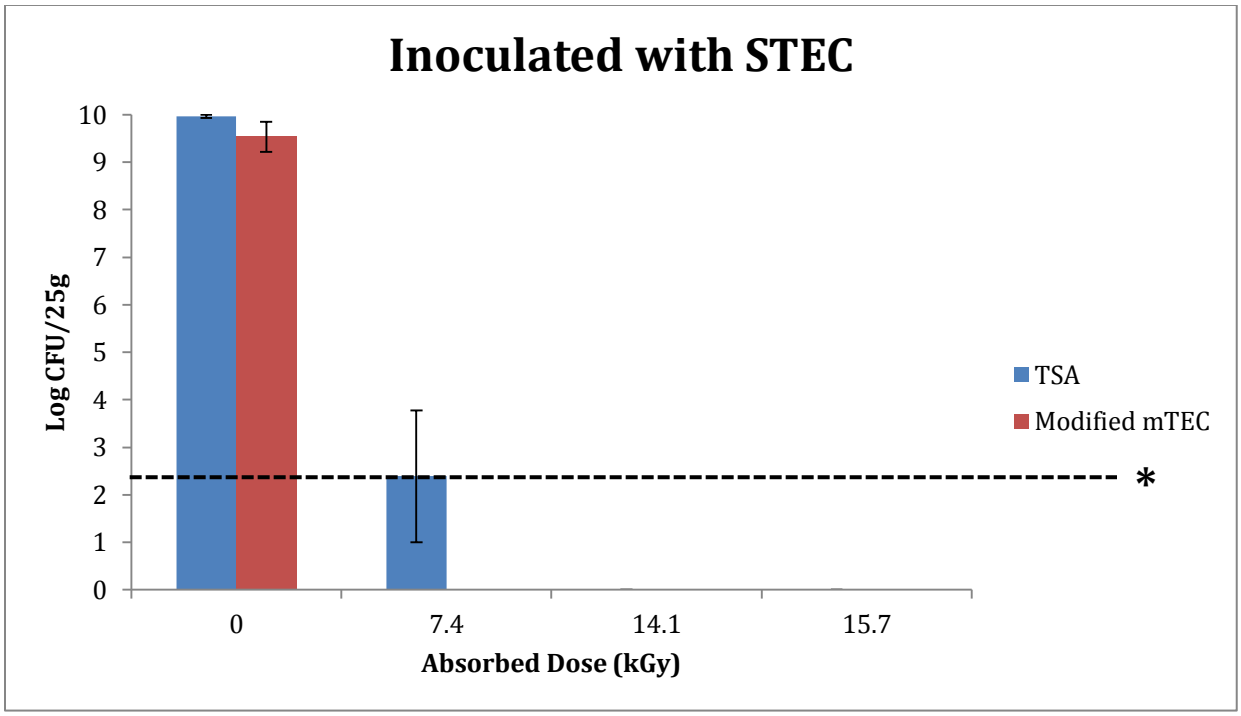
Table 3. Starting background aerobic bacterial populations

	Background Aerobic bacterial population (CFU/25g)
Dose response of *Clostridium sporogenes* spores	7.47
Shiga toxin producing E.coli (STEC) cocktail Trial 1	4.31
Shiga toxin producing E.coli (STEC) cocktail Trial 2	5.71
Clostridium sporogenes spore Trial 1	5.30
Clostridium sporogenes spore Trial 2	6.20
Accelerated Shelf Life Testing (ASLT)	4.54
AVERAGE	**5.59**
STANDARD DEVIATION	**1.16**

Sterility dose determination

The samples inoculated with the Shiga-toxin producing *E. coli* pathogen cocktail were initially exposed to target doses of 0, 8, 12, and 15 kGy. The absorbed (measured) doses were 0, 7.438, 14.135, 15.74 kGy. The starting bacterial population of the samples inoculated with the STEC

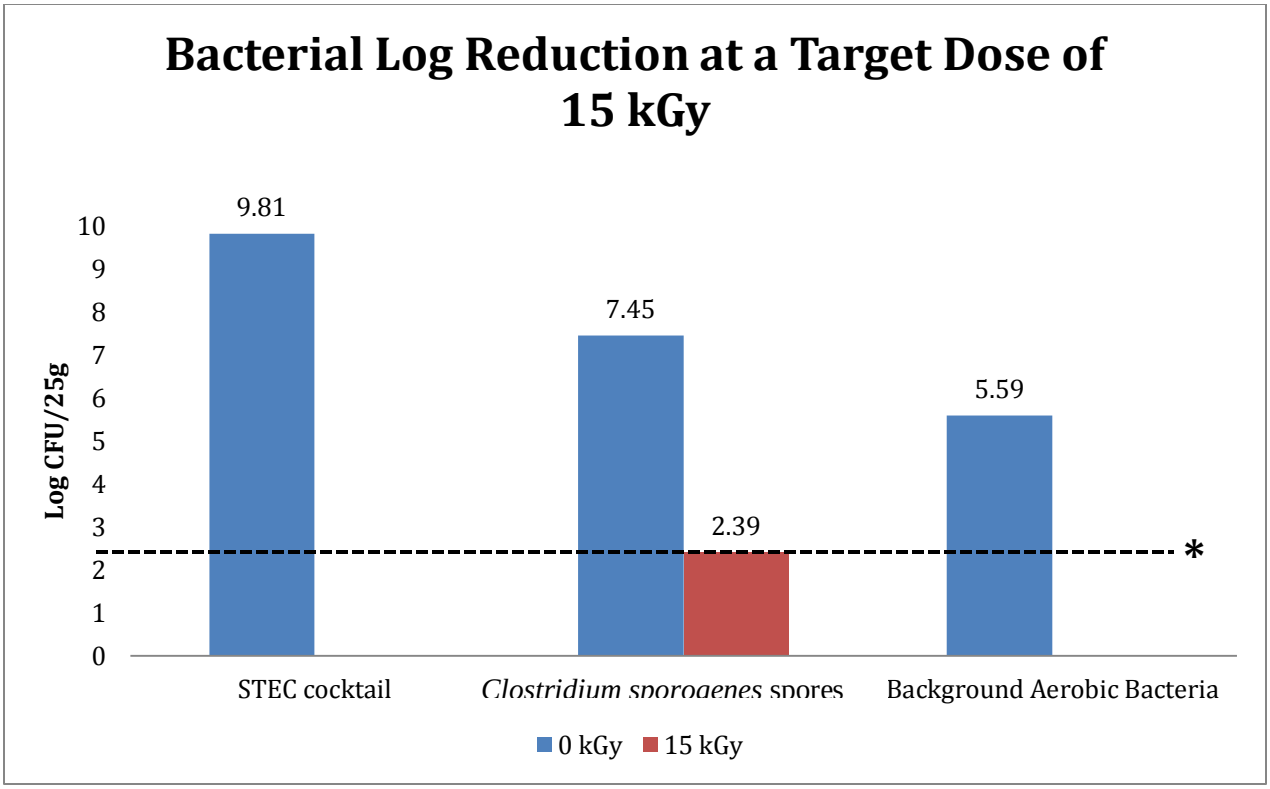
cocktail was approximately 10^{10} CFU/25 g. A dose of 7.4 kGy resulted in a 9.16 log reduction in the inoculated samples, but did not render the product sterile. Sterility was demonstrated at doses of 14.1 and 15.7 kGy (Figure 2). All proceeding experiments were carried out using 15 kGy as the hypothesized minimum sterility dose.



*Detection limit is 2.39 log CFU/25g.

Figure 2. STEC inoculated samples. Reduction in bacteria at various doses

The experiment was repeated with the retort pouches processed only at 0 and 15 kGy. In these trials, no growth was seen in any of 15 kGy processed products (Figure 3).



*Detection limit is 2.39 log CFU/25g. Absorbed dose for all STEC cocktail experiments was 16.0 ± 0.42 kGy. Absorbed dose for all *Clostridium sporogenes spore* experiments was 16.9 ± 0.45 kGy.

Figure 3. Effect of 15 kGy eBeam on various bacterial groups.

D10 determination of Clostridium sporogenes spores in Beef Fajitas

Samples inoculated with *C. sporogenes* spores were exposed to target doses of 0, 2, 4, and 8 kGy. Absorbed doses were 1.98, 3.81, 7.97 kGy. Using the slope of the regression line, the D10 value was calculated to be 2.61 kGy (Figure 4). With a calculated D10 of 2.61, a 15 kGy dose would achieve a 5.74 log reduction in spores. This is what was observed in this experiment. Holding the canning, or retort process, as the gold standard, approximately 31.32 kGy would be

needed to achieve a 12 log reduction in *Clostridium sporogenes* spores. More discussions need to be had in order to determine whether a 12 log reduction in spores is actually needed.

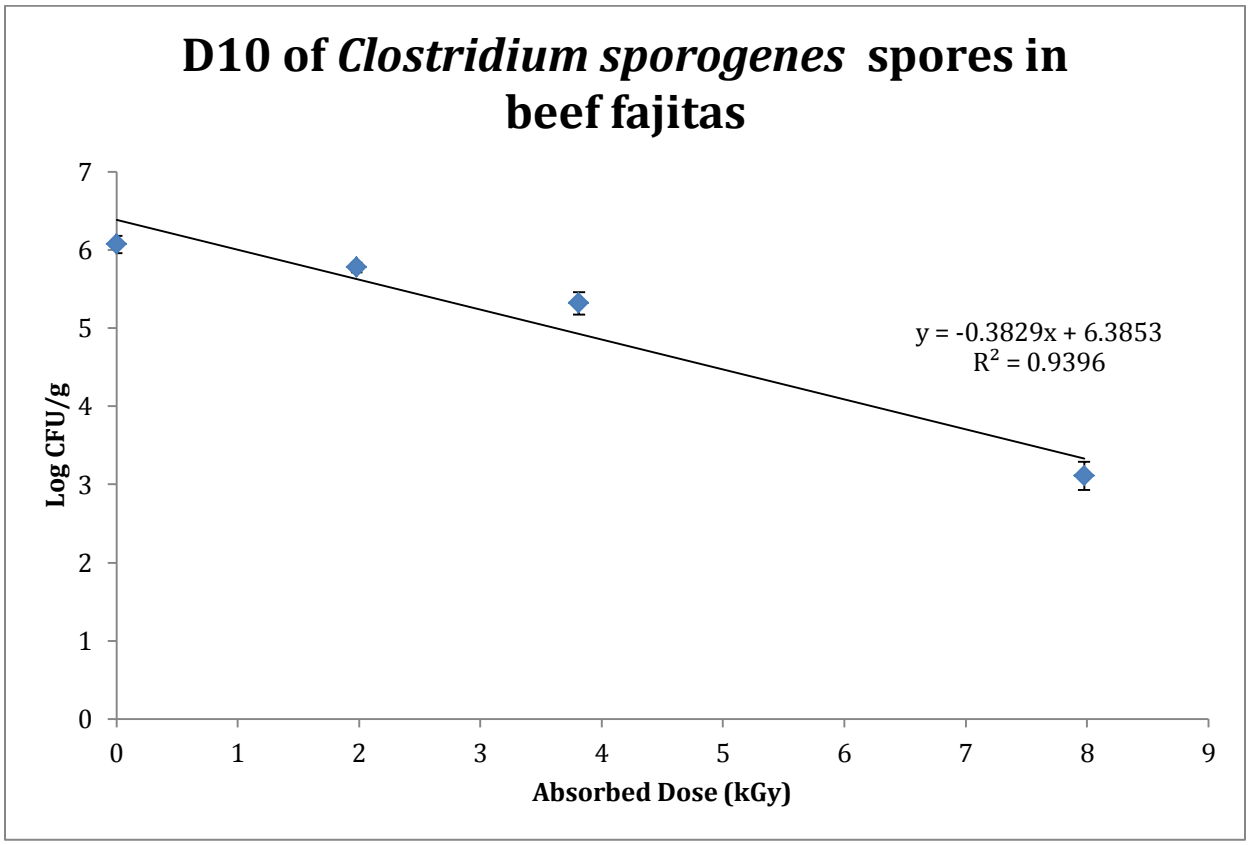


Figure 4. D10 of *Clostridium sporogenes* in beef fajitas

Accelerated shelf life testing

Bacterial and pH analysis

All irradiated samples were shown to still be sterile, even after being held at various temperatures for one month. A change in pH was evident immediately after eBeam irradiation (Table 4). While no statistical significance was seen between the unirradiated samples and those

irradiated at 15 kGy, the pH of the 44 kGy products was lower than the unirradiated at 15 kGy samples ($p < 0.05$). After one month, there was no significant difference seen between the pH of the 15 and 44 kGy products at any of the temperatures. Unirradiated (0 kGy) samples were not kept for one month due to the assumption that any unprocessed product would deteriorate beyond recognition due to.

Table 4. ASLT bacteria and pH data

Target Dose (kGy)	**Initial**		**After 1 Month storage**					
	Aerobic bacteria (CFU/25g)	**pH**	**4°C**		**25°C**		**55°C**	
			Aerobic bacteria (CFU/25g)	**pH**	**Aerobic bacteria (CFU/25g)**	**pH**	**Aerobic bacteria (CFU/25g)**	**pH**
0	4.54 ± 0.07	5.73 ± 0.07	-	-	-	-	-	-
15[a]	ND	5.70 ± 0.05	ND	5.84 ± 0.05	ND	5.68 ± 0.14	ND	5.34 ± 0.08
44[b]	ND	5.48 ± 0.11	ND	5.80 ± 0.04	ND	5.73 ± 0.01	ND	5.29 ± 0.10

ND = None Detected. The detection limit in this experiment was 250 CFU/25g.
[a] Absorbed dose was 15.78 ± 1.05 kGy
[b] Absorbed dose was 47.57 ± 4.24 kGy

	Initial	After one month		
		4 °C	25 °C	55 °C
0 kGy		-	-	-
15 kGy				
44 kGy				

Figure 4. Visuals of beef fajita samples at the beginning and end of the shelf life study

From a purely visual perspective, there were noticeable differences in the beef fajitas (Figure 4). Immediately after irradiation, the 0 and 15 kGy samples were very similar in appearance, although there was more liquid in the 15 kGy samples. The 44 kGy samples had even more liquid and were lighter in color. The 4 °C and 25 °C samples of both doses were slightly less bright than the freshly irradiated samples, but overall, still maintained their appearance. The samples stored at 55 °C for one month were extremely red and the texture was very different from the initial beef sample. The meat did not hold together and there was significantly more liquid in the pouches. Processed beef products stored at lower, refrigerated temperatures (4 °C) have been shown to retain their color and texture (moisture) attributes throughout their shelf life,

while products stored at elevated temperatures have been shown to lose their moisture over time (Sepulveda et. al. 2003).

GC/MS – olfactory

A total of 121 volatile compounds were detected across the 27 samples analyzed (Appendix A). When analyzing the effect of dose (0, 15, and 44 kGy), significant differences in volatiles were seen in eight volatile compounds (Table 5). Concentrations of allyl methyl sulfide, allyl mercaptan, and carbon disulfide decreased with increasing eBeam dose, while concentrations of 1-propene, 1-(methylthio)-,Z-, Styrene, 1-propene, 1-(methylthio)-, 1-hexene, and dimethyl disulfide increased with eBeam dose. The compounds such as 1-hexene and dimethyl disdulfide have been shown to only appear in irradiated samples, which is a trend seen in these samples as well (Kwon 2008). Furthermore, an increase in dimethyl sulfide has been seen across a variety of irradiated meat products (Kwon, 2008; Lee and Ahn, 2003; Nollet, 2012). As seen in Table 6 some of the descriptors used to describe these volatile compounds are positive when describing beef (Allyl methyl sulfide, allyl mercaptan), other are negative (dimethyl disulfide).

Table 5. Least Squared Mean values of compounds with significant differences, by dose

Dose (kGy)	**Compound**							
	Allyl methyl sulfide	Allyl mercaptan	Carbon disulfide	1-propene, 1-(methylthio)-,(Z)-	Styrene	1-Propene, 3-(methylthio)-	1-hexene	Dimethyl disulfide
0	1.57 (A)	2.95 (A)	1.51 (A,B)	0 (A)	-1E-15 (A)	-1E-15 (A)	0 (A,B)	-2E-16 (A,B)
15	3.35 (A,B)	0.37 (B)	1E-16 (B)	3.035 (B)	2.345 (B)	0.37 (A)	-2E-16 (B)	2E-16 (B)
44	0.842 (B)	-1E-16 (B)	1E-16 (B)	4.49 (B)	0.66 (A)	3.9 (B)	2.04 (A)	1.34 (A)

*Values having different letter designations are significantly different

Table 6. Aroma compound descriptors of dose dependent volatiles

Compound	**Aroma Descriptor**	**Source**
Allyl methyl sulfide	Meaty, fishy	Rotsatchakul et. al. 2008
Allyl mercaptan	Meaty, bologna	Rotsatchakul et. al. 2008
Carbon disulfide	Soft fruity onion	Bazemore et. al. 2000
1-propene, 1-(methylthio)-,Z-	-	-
Styrene	Sweet, balsamic, floral - extremely penetrating	Kerth and Miller 2015
1-Propene, 3-(methylthio)-,E-	-	-
1-hexene	-	-
Dimethyl disulfide	Asparagus-like, putrid	Kerth and Miller 2015

The basic assumption can be made that an increase in holding temperature increases the rates of degradation reactions, thereby allowing the various holding temperatures to act as time markers: The higher the holding temperature, the longer the simulated age of the product. While acetic acid concentration increased over the one month period for products treated with 15 kGy and 44 kGy, the increase was much larger for the 44 kGy product (Table 7). This increase in acetic acid may have been responsible for the decrease in pH seen in this treatment group. The concentration of compound 1- octane was significantly higher in the 44 kGy –55 °C products than any of the other treatment groups. Furthermore, 2-methyl-Furan and 2-furanmethanol may potentially be used as chemical markers of spoilage in this product, due to their significantly greater concentrations in samples stored at 55 °C. Dimethyl sulfide deviates from this pattern, and is only seen in significantly different amounts in the 44 kGy product that was held at 55 °C. As shown in Table 8, dimethyl sulfide has been described as being sulfurous, asparagus-like, and putrid.

Table 7. Significantly different compounds across all treatments

Treatment		Immediately after eBeam			After one month					
	Dose	**0**	**15**	**44**	**15 kGy**			**44 kGy**		
	Temp	**-**			**4 °C**	**25 °C**	**55 °C**	**4 °C**	**25 °C**	**55 °C**
Compound	**Acetic Acid**	3.09 (A,B,C)	4.44E-16 (C)	2.29 (A,B,C)	0 (C)	1.57 (B,C)	3.26 (A,B)	0 (C)	0 (C)	5.40 (A)
	Trans-Propenyl Propyl Disulfide	3.22 (A,B)	4.5 (A)	2.23 (A,B,C)	4.22 (A,B)	4.34 (A,B)	-4.4E-16 (C)	2.69 (A,B,C)	1.45 (B,C)	7.21E-16 (A,B,C)
	3,4-Dimethylthiophene	1.01 (C)	4.48 (A,B)	2.53 (A,B,C)	2.56 (A,B,C)	4.44E-16 (C)	1.75 (B,C)	4.44E-16 (C)	1.42 (B,C)	5.46 (A)
	Benzaldehyde	1.41 (B)	1.62 (A,B)	4.44E-16 (B)	4.52 (A)	1.51 (A,B)	0 (B)	1.31 (B)	0 (B)	-8.32E-17 (B)
	1-Propene, 1-(methylthio)-, (Z)-	-4.4E-16 (C)	3.28 (A,B)	5.20 (A)	3.04 (A,B,C)	4.54 (A)	1.29 (B,C)	4.79 (A)	3.10 (A,B,C)	5.11 (A)
	1-Octene	0 (B)	1.70 (B)	0 (B)	-1.1E-16 (B)	1.50 (B)	0 (B)	0 (B)	0 (B)	4.95 (A)
	2-methyl-Furan	3.33E-16 (B)	-1.1E-16 (B)	0 (B)	2.22E-16 (B)	-1.1E-16 (A)	4.75 (A)	3.33E-16 (B)	3.33E-16 (B)	3.86 (B)
	1-Propene, 3-(methylthio)-	4.44E-16 (C)	-4.4E-16 (C)	5.34 (A)	0 (C)	-4.4E-16 (C)	1.48 (B,C)	1.76 (B,C)	3.62 (A,B)	5.37 (A)
	2-Furanmethanol	2.22E-16 (B)	1.11E-16 (B)	1.11E-16 (B)	-4.4E-16 (B)	0 (B)	3.42 (A)	-4.4E-16 (B)	0 (B)	3.38 (A)
	Furan	7.77E-16 (B)	-1.1E-16 (B)	4.44E-16 (B)	-1.1E-16 (B)	-3.3E-16 (B)	1.60 (B)	-2.2E-16 (B)	-3.3E-16 (B)	4.44 (A)
	Decane	2.22E-16 (C)	6.66E-16 (C)	2.60 (A,B,C)	1.36 (B,C)	4.94 (A)	2.22E-16 (C)	4.44E-16 (C)	1.44 (B,C)	3.12 (A,B)
	Dimethyldisulfide	-1.1E-16 (B)	-3.3E-16 (B)	8.88E-16 (B)	6.66E-16 (B)	-5.6E-16 (B)	-5.6E-16 (B)	7.77E-16 (B)	-5.6E-16 (B)	4.93 (A)

Table 8. Aroma descriptors for compounds from all treatments

Compound	**Aroma Descriptor**	**Source**
Acetic acid	Sour, vinegar	Kerth and Miller 2015
Trans-propenyl propyl disulfide	Onion	Ohta and Osajima 1992
3,4-dimethylthiophene	-	-
Benzaldehyde	Almond, nutty, woody	Kerth and Miller 2015
1-Propene, 1-(methylthio)-, (Z)-	-	-
1-octene	-	-
2-methylfuran	Burnt material, sweet	Clifford and Wilson 1985
1-Propene, 3-(methylthio)-E	-	-
2-furanmethanol	Brown, roasted, oak	Hoff et. al. 2012, Prida and Chatonnet 2010
Furan	Roasted coffee, burnt rubber	De revel et. al. 2004
Decane	Floral	Kim et. al 2000
Dimethyldisulfide	Sulfur, Asparagus-like, Putrid	Kerth and Miller 2015, Shepard et. al. 2013

Potential error

While ASLT can be used to extrapolate the estimated shelf life of a product, the underlying premise is that the rate of reactions that lead to the product spoilage can be increased by increasing the holding temperature, and this relationship can be modeled linearly. One potential problem that arises with this assumption, is that an increase in temperature may cause a phase change in some of the components of food. For example, lipids are more likely to be liquid at higher temperatures, which make them more susceptible to lipid oxidation. This would proportionately increase the rate of the reaction, leading to an underestimated shelf life.

Furthermore, for a more accurate shelf life estimation, products should be sampled more often. More holding temperatures could also have been used to give more accurate results.

SECTION IV

CONCLUSION

Minimum Sterility Dose

Preliminary data suggests that the minimum eBeam dose required to sterilize beef fajitas is 15 kGy, but this dose provides for approximately a measured 10 log reduction in STEC organisms, and a 5 log reduction in *Clostridium sporogenes* spores. If the goal of applying a sterilizing dose to food products to achieve a 12 log reduction in *Clostridium sporogenes* spores, a 15 kGy dose would not satisfy this specification. Detailed deliberations are needed to decide whether space food requires a 12-log reduction of spores. The eBeam technology can be tuned to achieve any desired log reduction of spores. However, what benefits do a 12-log reduction achieve compared to the loss of sensory and nutritional attributes that will result from this higher dose? These discussion will need to involve microbiologists, food scientists, nutritionists, behavioral scientists and human performance engineers.

ASLT

A total of 121 volatiles were detected across the 27 total samples analyzed. Of these, many were shown to be dependent on the dose applied to the product. The concentrations of many compounds such as 2-methyl-Furan, 2-furanmethanol, and dimethyl sulfide increased as the product deteriorated. An increase in dimethyl sulfide was only present in the products processed at 44 kGy, showing that decreasing the dose to 15 kGy has the potential to prevent the concentration of specific off-flavors during spoilage.

Furthermore, once the minimum dose required for sterility (be that 15 kGy or 35 kGy), a more detailed accelerated shelf life study can be conducted to more accurately estimate the shelf life of these products. Comparisons between thermostabilized products and eBeam irradiated products would also be beneficial.

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APPENDIX

Appendix A. OVERALL LIST OF COMPOUNDS IN ALL SAMPLES

Compound	Mean	Standard Error
Ethanol	2.57	0.48
1-Propanethiol	2.79	0.45
Ethyl Acetate	1.38	0.46
Heptane	0.88	0.36
Butanal, 3-Methyl-	0.9	0.37
Benzene	2.6	0.45
Sulfide, Allyl Methyl	2.09	0.49
2,4-Dimethyl-1-Heptene	0.52	0.29
Hexanal	2.05	0.48
Iso Amyl Alcohol	0.96	0.4
Nonane	1.68	0.47
.Alpha.-Pinene, (-)-	2.38	0.52
Thiophene, 2,5-Dimethyl-	1.76	0.45
Disulfide, Methyl Propyl	3.87	0.39
2-.Beta.-Pinene	0.96	0.39
Trans Propenyl Methyl Disulfide	0.34	0.23
Acetic Acid	1.71	0.47
.Beta.-Myrcene	3.64	0.49
Furan, 2-Pentyl-	2.55	0.48
.Delta.3-Carene	1.19	0.49
.Alpha.-Terpinene	1.24	0.37
Octanal	3.18	0.44
Dl-Limonene	6.01	0.1
Benzene, 1-Methyl-2-(1-Methylethyl)-	3.23	0.56
.Gamma.-Terpinene	1.85	0.44
1h-Indene	0.89	0.33
Benzene, 1-Methyl-4-(1-Methylethenyl)-	0.99	0.41
Disulfide, Dipropyl	3.84	0.52
Trans-Propenyl Propyl Disulfide	2.53	0.44

Linalool	1	0.33
Decanal	2.16	0.4
Naphthalene	1.72	0.41
Phenol, 5-Methyl-2-(1-Methylethyl)-	0.45	0.25
Allyl Mercaptan	0.51	0.28
3,4-Dimethylthiophene	2.12	0.47
Benzaldehyde	1.2	0.4
Carbon Disulfide	0.17	0.17
Acetic Acid, Ethyl Ester	1.91	0.49
.Alpha. Terpinene	1.63	0.41
2-Furancarboxaldehyde, 5-Methyl-	0.28	0.2
Benzene, 1-Ethynyl-4-Methyl-	0.3	0.21
Thiophene, 2,4-Dimethyl-	0.88	0.32
3-Cyclohexen-1-Ol, 4-Methyl-1-(1-Methylethyl)-	0.41	0.23
Benzeneethanol	0.58	0.27
.Alpha.-Terpinolene	1.29	0.39
Trisulfide, Dipropyl	0.49	0.27
.Alpha.-Copaene	1.5	0.41
Trans Propenyl Propyl Trisulfide	0.43	0.24
Caryophyllene	1.64	0.49
Methanethiol	0.59	0.28
Cyclopropane, Ethyl-	0.4	0.28
2-Propanone	2.07	0.48
Hexane	0.45	0.31
2-Butanone	1.15	0.42
1-Heptene	2.3	0.51
1-Propene, 1-(Methylthio)-, (Z)-	3.3	0.45
1-Octene	0.94	0.39
Octane	1.75	0.49
Toluene	0.84	0.4
2-Pentenal, 2-Methyl-	1.21	0.4
1-Nonene	1.12	0.41
1-Propene, 3,3'-Thiobis-	0.35	0.24

Styrene	1.36	0.38
O-Isopropenyltoluene	0.3	0.21
Hexanoic Acid	0.32	0.22
Azulene	0.71	0.33
Phenol	0.68	0.32
Methylsuccinic Anhydride	0.32	0.22
Pentane, 3-Methyl-	0.41	0.28
N-Propyl Cis-1-Propenyl Sulfide	0.83	0.34
1-Pentene, 4-Methyl-	0.34	0.24
1,3-Octadiene	0.43	0.24
Benzene, 1-Methyl-4-(1-Methylethyl)-	1.39	0.46
Nonanal	1.45	0.44
Furan, 2-Methyl-	0.99	0.41
1-Nonanol	0.27	0.19
1-Propene, 3-(Methylthio)-	1.82	0.5
Disulfide, Dimethyl	0.15	0.15
Benzene, Methyl-	0.4	0.28
2-Propylfuran	0.14	0.14
2-Butenal, 2-Ethyl-	0.12	0.12
2-Heptanone	0.75	0.31
Decyl Trifluoroacetate	0.29	0.2
2-Furanmethanol	0.79	0.37
Hentriacontane	0.29	0.2
Benzene, 1,3-Bis(1,1-Dimethylethyl)-	1.96	0.5
.Alpha.-Humulene	0.76	0.31
Furan	0.7	0.33
Thiophene, 2-Methyl-	0.38	0.27
Octanoic Acid, Ethyl Ester	1.5	0.45
Copaene	0.31	0.22
Phenol, 2-Methyl-5-(1-Methylethyl)-	0.46	0.25
Methane, Thiobis-	0.38	0.26
Benzene, 1-Methyl-3-(1-Methylethyl)-	0.32	0.22
Trans-Caryophyllene	0.34	0.23

Isopropylcyclobutane	0.92	0.38
Octane, 4-Methyl-	1.51	0.42
3-Ethyl-3-Methylheptane	0.29	0.2
Decane	1.45	0.44
Undecane, 4,7-Dimethyl-	0.35	0.24
1-Decene	1.18	0.43
Heptanal	0.69	0.33
Ethanone, 1-Phenyl-	0.27	0.19
Nonahexacontanoic Acid, Methyl Ester	0.38	0.21
Decanoic Acid, Ethyl Ester	0.68	0.32
N Heptanal	0.56	0.31
3-Phenylpropyne	0.3	0.21
2-Ethyl Crotonaldehyde	0.29	0.20
Benzene, Methyl(1-Methylethyl)-	0.36	0.25
1-Hexene	0.86	0.35
1-Propene, 3-[(1-Methylethyl)Thio]-	0.55	0.31
Thiazole, 2-Ethyl-	0.39	0.22
Propanal, 2-Methyl-	0.34	0.24
Trans-1-Butyl-2-Methylcyclopropane	0.4	0.28
2 Methyl 2 Pentenal	0.35	0.24
2(3h)-Furanone, Dihydro-	0.33	0.23
1-Tridecene	0.48	0.27
Dodecanal	0.27	0.18
Dimethyldisulfide	0.57	0.32
Undecane, 5,7-Dimethyl-	0.28	0.19
Carbonochloridic Acid, Decyl Ester	0.32	0.22
Thiazole, 2-Nitro-	0.33	0.24