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Individual and combined efficacies of mild heat and ultraviolet-c radiation against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in coconut liquid endosperm

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ABSTRACT

This study determined the inactivation kinetic parameters of selected pathogens in heat, ultraviolet-C and combined heat-UV-C treated coconut liquid endosperm. Separate cocktails of *Escherichia coli* O157:H7, *Salmonella enterica* serovars, and *Listeria monocytogenes* strains were inoculated into coconut liquid endosperm (pH 5.15, TSS 4.4°Bx, TA 0.062% malic acid, extinction coefficient (ε) at 254 nm of 0.0154 cm^{-1}) for inactivation studies. Result showed that all organisms generally exhibited a log-linear heat inactivation behavior (R^2 0.81–0.99). The *E. coli* O157:H7 cocktail (D_{55} = 19.75 min, D_{57} = 10.79 min, D_{60} = 3.38 min, and D_{63} = 0.46 min) was found to be significantly more resistant ($P > 0.05$) than the tested cocktail of *L. monocytogenes* (D_{55} = 11.68 min, D_{57} = 4.53 min, D_{60} = 1.82 min and D_{63} = 0.26 min) and *S. enterica* cocktail (D_{55} = 3.08 min, D_{57} = 2.60 min, D_{60} = 0.89 min and D_{63} = 0.25 min). Despite the differences in D_T values, computed z values for *L. monocytogenes* cocktail (5.12 ± 0.43 °C) and *E. coli* O157:H7 cocktail (4.95 ± 0.12 °C) were not significantly different ($P > 0.05$), but were both significantly ($P < 0.05$) lower than that of *S. enterica* cocktail (7.10 ± 0.15 °C). All test organisms also exhibited a generally log-linear UV-C inactivation behavior (R^2 0.90–0.99) with *E. coli* O157:H7 cocktail (D_{UV-C} = 25.26 mJ/cm^2) demonstrating greatest resistance to UV-C than *S. enterica* (D_{UV-C} = 24.65 mJ/cm^2) and *L. monocytogenes* (D_{UV-C} = 17.30 mJ/cm^2) cocktails. The D_{55} values of each organism cocktail were used to calculate for the 3-log reduction heating process schedules, during which UV-C treatments were simultaneously applied. Lethal rates (*F* values) calculations in the combined processes revealed that within the 3-log reduction heating processes, co-exposure of UV-C resulted in 5.62 to 6.20 log reductions in the test organism populations. Heating caused 69.3, 97.2, and 67.4% of the reduction in *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* cocktails, respectively. These results can be used as baseline data in the establishment of mild heat treatment in combination with UV-C process schedules for coconut liquid endosperm and other similar products.

1. Introduction

According to the Philippine Coconut Authority (PCA) (Forbes, 2013) and the United Nations Conference on Trade and Development (UNCTAD, 2012), the Philippines ranks as one of the leading producers of coconuts in the world. Based on the sales recorded in the year 2011, the value of coconut products that the Philippines shipped out reached about $2 billion due to its strong market demand (Alave, 2011). Coconut liquid endosperm beverage is one of the products that have benefitted from this increase in market demand. It is now a highly valued product and is claimed as a natural contender in the sports drink market because of its delicate aroma, taste, nutritional and therapeutic properties attributed to its unique chemical composition of sugars, vitamins, minerals, amino acids, and phytohormones (FAO, 2005; Yong et al., 2009). The Philippine National Standard for young coconut liquid endosperm or coconut water published by the Bureau of Product Standards (BPS, 2006) defines coconut liquid endosperm as natural aqueous liquid of 6–9 month-old coconut drupes, the appearance of which ranges from clear to slightly turbid.

Coconut liquid endosperm is usually consumed fresh and

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unprocessed by most of the consumers (Gabriel et al., 2016). This may be attributed to the shift in consumer preferences towards healthier food products that are minimally processed (Gabriel, 2014). However, due to its inherent characteristics, microbial contamination may occur once the coconut liquid endosperm is extracted from its drupe and exposed to external environment (Rolle, 2007). In a study conducted by Reddy (1995), the traditional, manual collection of coconut liquid endosperm introduced microbial contamination level of up to 6.0 log CFU/ml, which may include both spoilage and disease-causing microorganisms such as *Salmonella*, *Bacillus cereus*, and *Staphylococcus aureus* (Leite et al., 1998; Melo et al., 2003) and *Listeria monocytogenes* (Walter et al., 2009).

Non-pasteurized fruit juice consumption has been implicated with occurrences of foodborne illnesses involving pathogens such *Escherichia coli* O157:H7 and *Salmonella enterica* (Vojdani et al., 2008). *Listeria monocytogenes* has not yet been involved in outbreaks but food safety advocates as well as regulatory bodies agree that the risk this pathogen poses to fruit juices should also be given attention due to its pathogenicity, particularly towards susceptible population groups including children, pregnant, and immunocompromised individuals (Gabriel and Estilo, 2015; Federal Register, 2001; Kathariou, 2002). Furthermore, the inherent resistance of *L. monocytogenes* towards conditions that are not easily tolerated by pathogens such as *E. coli* O157:H7 and *S. enterica* make *L. monocytogenes* an organism of public health significance in fruit juice products (Gabriel and Nakano, 2010).

As a result, the United States Department of Agriculture (USDA) and United States Department of Health and Human Services (USDHHS) (2001) ratified the Hazard Analysis Critical Control Point (HACCP) for juice manufacturers that requires the application of a process or series of processes that will result in a 5-log reduction (99.999%) of the pertinent pathogen in the juice being processed using traditional and approved novel food technologies. Thus, the required level of lethality may be achieved through the use of different unit operations during fruit juice manufacture with respective bactericidal outcomes.

Thermal pasteurization is the most commonly applied processing technique to inactivate spoilage microorganisms and harmful pathogens in raw materials (Mak et al., 2001). However, heat treatment can cause a significant reduction in physical, nutritive and sensory quality of foods. Flavor changes in foods due to heating have been reported by many studies (Bell and Rouseff, 2004; De Roeck et al., 2010; Reddy and Love, 1999; Shaw, 1982; Shreirer et al., 1977) limiting the product's marketability (Damar, 2006). Thus, because of the increasing consumer demand for fresh, high quality and nutritious food products, considerable interest for alternative processing technologies with adequate microbial inactivation potential has provided an impetus towards further research in non-thermal processing technologies (Patil et al., 2009).

The term novel non-thermal processing is often used to designate non-traditional technologies that have the ability to inactivate microorganisms, such as pulsed electric field, pulsed light, ozone and ultraviolet irradiation (Butz and Tauscher, 2002). UV technology is considered one of the effective means of disinfection, which excludes the necessity of heat to inactivate microorganisms (Sastry et al., 2008). The use of ultraviolet light at germicidal wavelengths has been approved to treat food surfaces and clear fruit juices (US-FDA, 2002). However, the efficiency of UV-C radiation depends on the UV-C absorption of the food matrix (Guerrero-Beltran and Barbosa-Canovas, 2005; Koutchma, 2014; Koutchma et al., 2007; Lopez-Malo and Palou, 2002). Hence, the concept of combining several factors (hurdles) to preserve foods has been developed by many researchers since their expected combined use will have a greater efficacy in inactivating microorganisms than the use of just a single factor (Gabriel and Estilo, 2015). Leistner and Gould (2002) explained that hurdle technology employs a deliberate and intelligent consideration and manipulation of several preservative factors or techniques to control microorganisms in foods. Hurdle technology has gained popularity in the past couple of decades due to its multi-target approach in controlling microorganisms in food. Microorganisms are inactivated from the accumulation of various injuries due to several mild inactivating factors, and not just from a single injury due to a single severe inactivating factor.

It was therefore the aim of this study to determine and compare the inactivation kinetic parameters of cocktails of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in coconut liquid endosperm using heat, continuous ultraviolet-C and combined heat and UV-C treatments. Specifically, the study aimed to establish the inactivation kinetic parameters of the test organisms in coconut liquid endosperm at several test temperatures (55, 57, 60, and 63 °C); and in UV-C-treated suspending medium. This study also determined the combined lethal rates (*F*-value) of heat and UV-C against the test organisms in coconut liquid endosperm. Result of this study will provide information in the selection of test pathogens that can be used in future challenge studies and will guide manufacturers in the establishment of the appropriate processes and schedules for coconut beverage and similar products.

2. Materials and methods

2.1. Coconut liquid endosperm sample

All inactivation studies were conducted in a commercial coconut liquid endosperm suspending medium (Nyogi, Batch L4, Pure Coconut liquid endosperm, Philippine Island for My Philippines Lifestyle Inc. Col., Matalino St. Quezon City, Metro Manila). The experiment setups are illustrated in Fig. 1. Coconut liquid endosperm from the same production batch were used for all inactivation experiments to minimize variation in composition and/or physicochemical characteristics. The coconut liquid endosperm had a total soluble solid (TSS) of 4.4 °Brix, pH value of 5.15 and titrable acidity (TA) of 0.062% malic acid which were measured using a hand held refractometer (Atago, Japan), pH meter (Eutech pH 700, Singapore) and titration method (AOAC, 2007), respectively. The UV-absorbance of the coconut liquid endosperm was also characterized (Fig. 2) using a spectrophotometer (Unico 4802 UV/Vis Double Beam Spectrophotometer, USA). The coconut liquid endosperm used in this study had a UV-C absorbance of 1.9 (UV-C transmittance of 1.26%) and an extinction coefficient (ε) at 254 nm equal to 0.0154 cm^{-1}. Prior to being used in the experiments, the orange juices were analyzed for background microflora. The aerobic bacteria and yeast and mold counts were below detection limit (< 1.0 log CFU/ml).

2.2. Test organisms

In this study, five strains of *E. coli* O157:H7, seven strains of *S. enterica*, and two strains of *L. monocytogenes* were challenged. The five *Escherichia coli* O157:H7 strains were from the Laboratory of Food Microbiology and Hygiene, University of the Philippines Diliman (LFMH)-maintained cultures (EMY-10, EDT-10, EMN-10, ECR-10, and EHC-10). The seven *Salmonella enterica* strains included serovars Typhimurium (American Type Culture Collection, ATCC 14028), Diarizonae (ATCC 12325 and 29,934), Abortus-Equi (ATCC 9842), Enteritidis (LFMH S1–10), Infantis (LFMH S2–10), and Montevideo (LFMH S3–10). Two *Listeria monocytogenes* strains, namely 1/2c (LFMH L1–10) and 4b (LFMH L2–10) were also combined at an equal ratio prior to inoculation and subsequent challenge studies. All LFMH bacterial strains were previously isolated from food and environment samples.

Culture slants were prepared by obtaining a loopful of inoculum from each nutrient agar (NA, Hi-Media, Mumbai, India) stock culture slants, and subculturing by transferring into 10 ml nutrient broth (NB, Hi-Media, Mumbai, India) and incubating at 37 °C for 24 h. Each of the overnight cultures was separately enriched by transferring loops of inoculum to a new set of NB tubes and incubating for another 24 h.

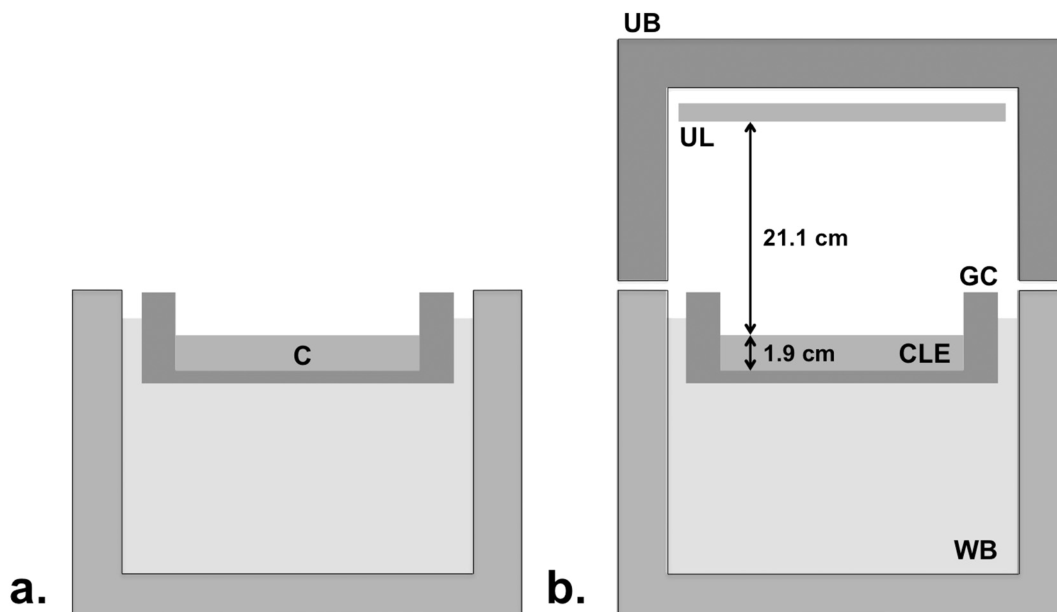


Fig. 1. Experiment setups for the thermal (a) and UV-C (b) inactivation studies. WB: water bath, UB: fabricated UV-C box; UL: 15 W UV lamp; GC: glass container for CLE: coconut liquid endosperm. C in (a) indicates the approximated location of the geometric center where temperature monitoring during thermal inactivation studies.

Culture slants were finally prepared by transferring loops of inoculum from each enriched cultures into NA slants, and incubating for another 24 h. The culture slants were subjected to refrigerated storage until use.

2.3. Cocktail inoculum preparation

In the preparation for cocktails of inoculum, cells from the culture slants were prepared by subjecting each of the test organism strains to the previously described protocols. After the sequential subculture and enrichment in NB, 1 ml of aliquot was obtained from each culture tube. Cells from the same genus were then combined in a sterile tube and vortexed to ensure homogeneity. The cocktailed cells were harvested by spinning several 1 ml aliquots of culture media (placed in Eppendorf tubes) in a bench top centrifuge (Cole Parmer, USA) at 6000 rpm for 15 min. The supernatant was then discarded and the cell pellets left were re-suspended and acclimatized in coconut liquid endosperm at ambient conditions for 10 min prior to challenge studies. The acclimatized cell suspensions were then pooled in a sterile flask and immediately used in the challenge studies.

2.4. Heat challenge study

The inactivation studies were conducted following methods described by Gabriel et al. (2015) with minor modifications. Two uninoculated 250 ml of coconut liquid endosperm samples were aseptically placed in two separate rectangular container (17 cm × 11.5 cm × 6 cm). The first container was inoculated with 2.5 ml aliquot of the acclimatized cocktail cell suspension with approximately 7.0 log CFU/ml. The unheated suspension was mixed before 0.1 ml was pipetted out and surface-plated onto the NA plates (reported as time zero, t = 0). The second container with uninoculated sample was immersed in a digital water bath (Lab Companion, Batch Circulator, Jejo Tech, Korea) and allowed to equilibrate at desired heating temperature (55, 57, 60, or 63 °C). The suspending medium temperature was measured by inserting a thermometer through the cold point of the container. When the cold point temperature reached the

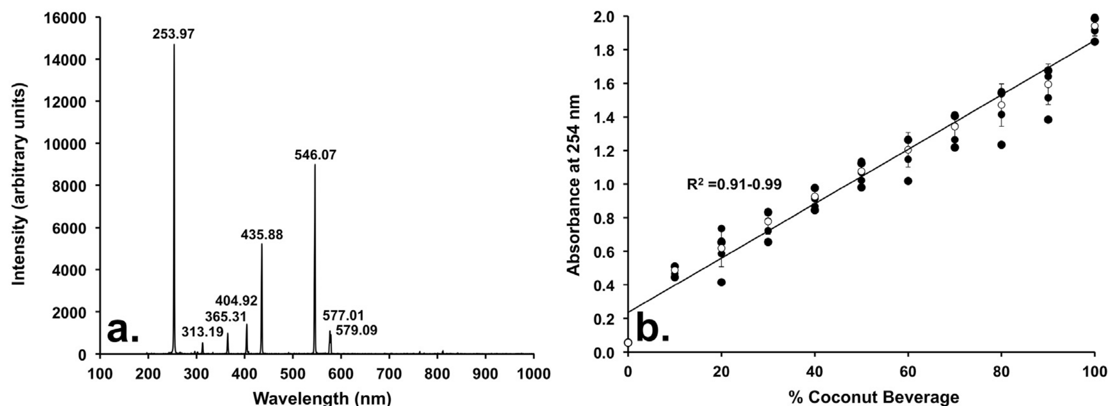


Fig. 2. Optical characteristics of UV-C lamp and coconut liquid endosperm suspending medium. (a) Emission spectra of the 15-w UV-C lamp source showing dominant emitted radiation at 254 nm. (b) Coconut liquid endosperm concentration vs. absorbance plot from which the UV-C absorption coefficient (ε) was measured. Black markers represent values obtained from internal and external replications; white markers and error bars represent average values and standard deviations calculated from the four data points, respectively. The R^2 ranges represent the coefficients of determination obtained from the 4 replications. External replications represent independent experiment runs. Internal replications refer to duplicate analyses per experiment run.

desired temperature, 2.5 ml aliquots of the acclimatized cocktail cell suspension was inoculated to introduce approximately 7.0 log CFU/ml cells. The inoculated suspending medium was constantly, manually agitated throughout the heating process. A sample from the heated inoculated suspending medium was pipetted out at predetermined exposure time intervals and was immediately transferred in tubes immersed in an ice bath for not longer than 10 min. Tubes containing the heat-treated inoculated coconut liquid endosperm were immediately subjected to serial 10- fold dilutions with 0.1% peptone water (Hi-Media, India) and surface-plated onto NA plates (HiMedia, India). All plated *E. coli* O157:H7 and *S. enterica* serovars dilutions were incubated at 35 °C for 24 h while *L. monocytogenes* was incubated at room temperature for 48 h before survivor enumerations.

2.5. Ultraviolet-C challenge study

Acclimatized cells (7.0 log CFU/ml) were immediately introduced into 250 ml of coconut liquid endosperm (1.9 cm thickness) in a rectangular glass container (17 cm × 11.5 cm × 6 cm) and was exposed to UV-C radiation using a fabricated UV-C box with a mounted UV light source (15 W) lamp characterized by a predominant emission of 254 nm at a surface irradiance of 0.42 mW/cm^2 measured using UVX radiometer (Ultra-Violet Products Ltd., Cambridge CB4 1TG UK). The UV-C source was previously subjected to optical emission spectroscopy by Gabriel et al. (2016) that confirmed predominant radiation of 254 nm (Fig. 2). Measurement was done using a Spectrometer (Ocean Optics, Inc., FL., USA) with a dispersion of 0.2467 nm per pixel and an optical resolution of 1.09 nm in the range of 200–1100 nm.

The UV-C box was positioned at the top of the unheated water bath with a lamp-to sample distance of 21.1 cm. The inoculated sample was constantly and manually agitated throughout the exposure time. The UV-C treatments were conducted at ambient temperature (25 °C) and the maximum exposure times range from 50 to 125 s. After exposure to a predetermined time intervals, the UV-C is turned off allowing for the withdrawal of the treated samples. Samples were then subjected to serial 10-fold dilution with 0.1% peptone water (HiMedia, India), after which 0.1 ml was obtained from appropriate dilutions and spread-plated on nutrient agar (NA, HiMedia, India). All plated *E. coli* O157:H7 and *S. enterica* serovars dilutions were incubated at 35 °C for 24 h while *L. monocytogenes* was incubated at room temperature for 48 h before survivor enumerations.

2.6. Survivor enumerations, determination of inactivation kinetic parameters

The enumerated survivor populations of test organisms were expressed as log CFU/ml. The death kinetic parameters were characterized using linear regression. Alabastro (1987) explained that the thermal decimal reduction time (D_T value) measures the resistance of the test organism to an inactivating agent and is defined as the unit time of exposure of the cells to a heating temperature that shall result in 1 log reduction (90%) in the initial population. The D_T value was equivalent to the negative inverse of the slope of the survival curve. Similarly, the thermal resistance constant (z value) is equivalent to the increase in the heating temperature needed to reduce the D_T value by 10-fold, or increase the process lethality by 10-fold. The z value was determined by obtaining the negative inverse of the slope of the log of D_T values against temperatures. The $D_{UV\text{-}C}$ values of the test organisms were determined as the negative inverse of the slope of the survivor curve, and were defined as the UV-C energy dose (mJ/cm^2) necessary to reduce the initial population of a test organism by 1-log. In this study only inactivation curves that traversed > 1 log were used in the calculation of inactivation kinetic parameters.

2.7. Combined heat and UV-C challenges

Each of the cocktails of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* were subjected to simultaneous heat and UV-C treatments. In the measurement of the combined efficacies of heating and UV-C against each of the test species, the time required to achieve a 3-log reduction at 55 °C ($3D_{55}$) was calculated per challenge organism. The $3D_{55}$ process schedule was determined by multiplying the thermal D_{55} value of each organism with 3 to come up with a 55 °C heating process capable of reducing the target organism by 3 logs (99.9%). In the combined challenges, the set up used in the UV-C inactivation was similarly employed (Fig. 2) except that the water bath was heated to the desired temperature (55 °C). Acclimatized cocktail cell suspensions were inoculated into 250 ml coconut liquid endosperm in order to have an initial population of approximately 8 log CFU/ml. The coconut liquid endosperm suspension was manually agitated and after which a sample was pipetted out and surface-plated onto NA plates (t = 0).

The inoculated liquid suspension was then immersed in a water bath until it reached the desired sample temperature. Heat penetration within the inoculated sample was conducted by measuring the temperature in the cold point every 1 min. Come up time ranged from 10 to 12 min. When the desired heating temperature (55 °C) was reached the UV-C lamp was turned on to expose the inoculated medium to irradiance similarly employed in the individual UV-C challenge (0.42 mW/cm^2). The inoculated sample was exposed to the combined heating at 55 °C and UV-C for the duration of the calculated $3D_{55}$ process schedule specific for the challenge organism. The UV-C lamp was immediately turned off, and the heated sample was cooled in an ice bath until it reached ambient temperature. One ml aliquots were obtained from the treated samples to enumerate survivor populations and calculate for the total log reductions due to the combined heat and UV-C treatments.

2.8. Determination of lethal rates due to heat during combined heat and UV-C challenges

To determine the individual contribution of heat and UV-C during the simultaneous challenge studies, the lethal rate for a given heating time interval, *L*, due to heat was calculated using Eq. (1), where T is the temperature (°C) at time t, and T_r is the reference temperature (55 °C) and z is the heat resistance parameter (z-value) computed for each test pathogen.

$$L = 10^{\frac{(T-Tr)}{z}} \tag{1}$$

Based on the Simpson's Rule (Da-Wen Sun, 2012; Gabriel, 2015a; Salleh-Mack and Roberts, 2007), the total lethality for the test organism throughout the treatment period, *F*, was then determined using Eqs. (2)–(3), where t is the time at a target process temperature.

$$F = \int_0^t Lt\ dt \tag{2}$$

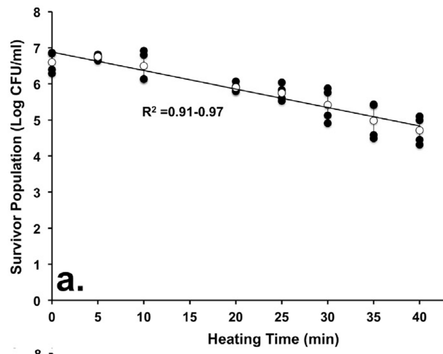
$$F = \sum_0^t L\ \Delta t \tag{3}$$

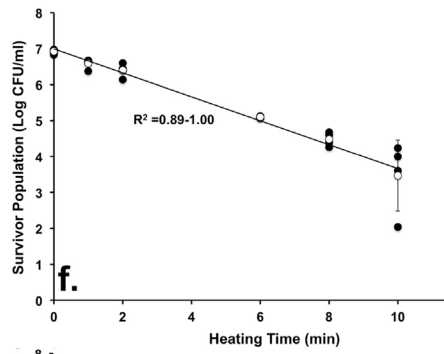
Finally, the heat-mediated reduction of test organisms in the combined heat-UV-C treatment coconut liquid endosperm was determined using Eq. (4), where N_0 is the initial population, and D is the decimal reduction time at 55 °C.

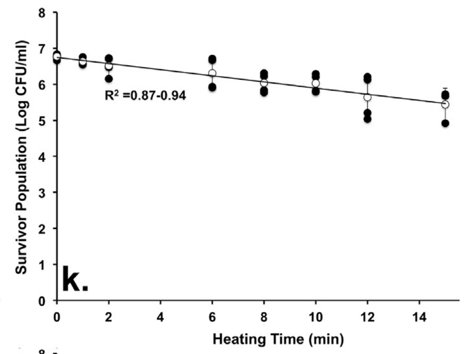
$$\log_{10}\left(\frac{No}{N}\right) = \frac{F}{D} \tag{4}$$

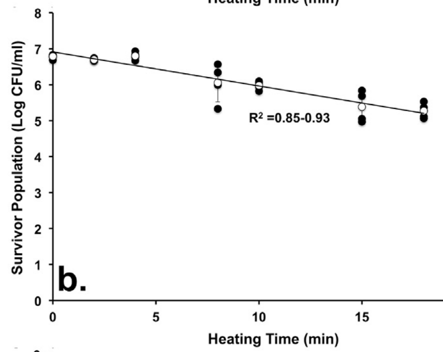
2.9. Statistical analyses

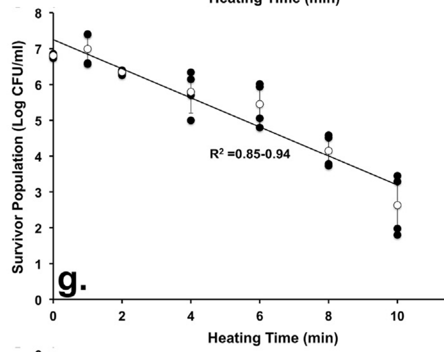
All inactivation studies were conducted four times, distributed in two independent experiment runs. Data obtained from independently

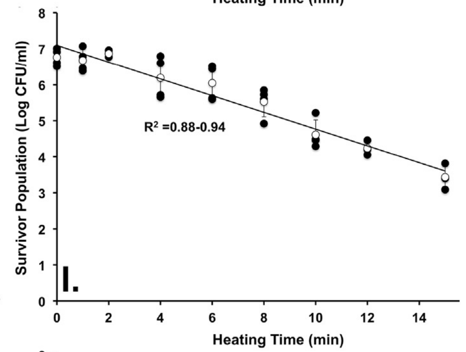


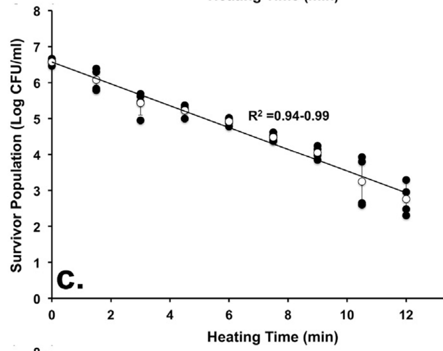


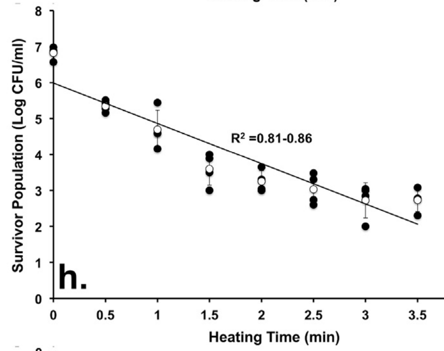


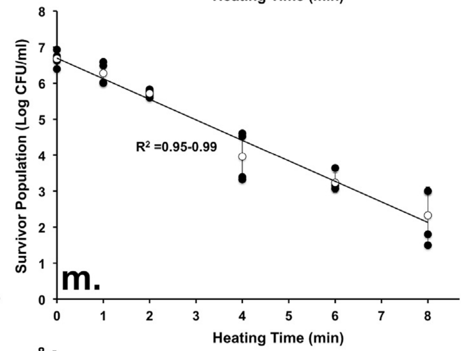


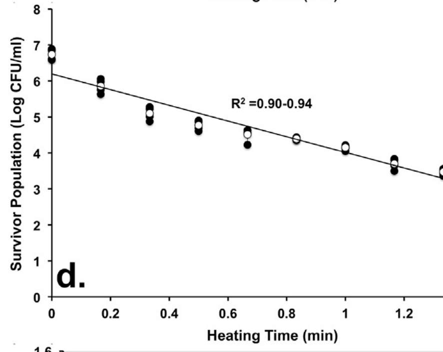


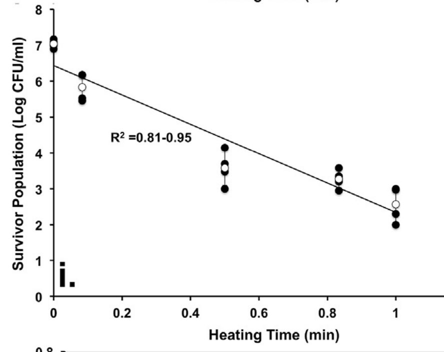


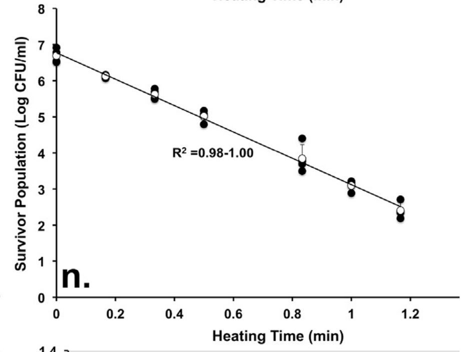


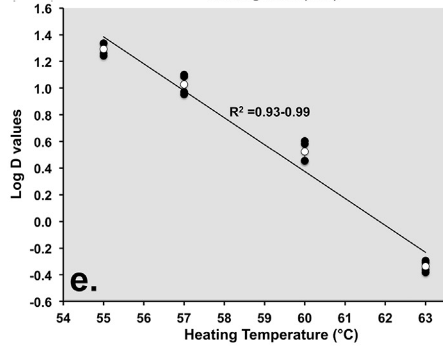


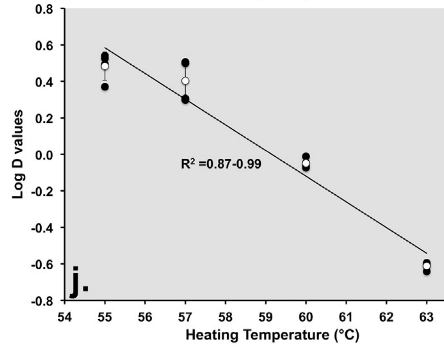












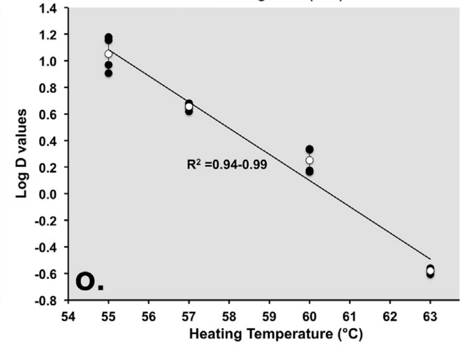


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Fig. 3. Determination of thermal resistance parameters of *E. coli* O157:H7, *S. enterica*, and L. *monocytogenes* in coconut liquid endosperm. Inactivation curves of *E. coli* O157:H7 at 55, 57, 60, and 63 °C (a–d) where decimal reduction times (D_T values) were calculated from. Log D_T values vs. heating temperatures plot (e) from which z value of *E. coli* O157:H7 was obtained. The D_T values and z values of *S. enterica* were calculated from (f–i) and (j), respectively. The D_T values and z values of L. *monocytogenes* were calculated from (k–n) and (o), respectively. Black markers represent values obtained from internal and external replications; white markers and error bars represent average values and standard deviations calculated from the four data points, respectively. The R^2 ranges represent the coefficients of determination obtained from the 4 replications. External replications represent independent experiment runs. Internal replications refer to duplicate analyses per experiment run.

replicated experiments were subjected to single factor analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) of the SAS Statistical Software Package version 8.0 (Cary, NC, USA). The Duncan's multiple range test (DMRT) was used for post-hoc determinations of significant differences at 95% level of significance.

3. Results and discussion

3.1. D_T- and z-values of test pathogens in heated coconut liquid endosperm

Results showed that all test cocktails of organisms generally exhibited a monophasic inactivation curve in the heated suspending medium. The log-linear thermal inactivation curves of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* cocktails in coconut liquid endosperm are presented in Fig. 3**.** A log-linear inactivation curve is a hallmark of a first-order death kinetics that was similarly described by Moats (1971) for the thermal inactivation of microorganisms in food matrices. Peleg (2006) explained that non-linear inactivation curves with downward or upward concavity indicate non-homogenous efficacy of the inactivating agent towards populations being tested. A downward concavity may indicate the need for injury to accumulate before cells are inactivated. Upward concavity in the inactivation curve may indicate that the challenge organism cocktail is composed of a mixture of cells with varying resistance. Nonlinearity in inactivation curves may be attributed to several factors including the physicochemical properties of the suspending medium and inherent resistance of the treated organisms.

As expected, decreasing D_T values, were observed as the heating temperature increased, indicating greater cell death per unit time of exposure. Table 1 shows that among the pathogens that were subjected to thermal inactivation, the cocktail of *E. coli* O157:H7 cells had significantly higher heat resistance ($P < 0.05$) at all temperatures, followed by *L. monocytogenes* and *S. enterica* cocktail. However, the D_{63} values of *L. monocytogenes* and *S enterica* cocktails were found to be not significantly different ($P > 0.05$). Table 1 further shows that increasing the temperature by 2–3 °C significantly decreased the thermal resistance ($P < 0.05$) of *E. coli* O157:H7. However, in the case of *L. monocytogenes* and *S. enterica* cocktails increasing the temperature did not significantly decrease their thermal resistance ($P > 0.05$).

Results revealed that although increasing the temperature from 55 to 60 °C significantly decreased the thermal resistance ($P < 0.05$) of the cocktail of *L. monocytogenes*, non-significant ($P > 0.05$) decrease in thermal resistance was observed at 60 and 63 °C. On the other hand, no significant difference ($P > 0.05$) in the computed decimal D_T values of *S. enterica* cocktail at 55 °C and 57 °C, but elevating the temperature to 60 °C and 63 °C significantly decreased D_T values ($P < 0.05$). Furthermore, the D_{60} and D_{63} values of the cocktail of *S. enterica* were found to be not significantly different ($P > 0.05$). The relatively less variation in the D_T values of the tested cocktail of *S. enterica* within the heating temperature range tested translated to the observed trend in the z values where *S. enterica* was found to have significantly greater thermal resistance constant (z-value) ($P < 0.05$) than the other two tested organisms. These observations are in agreement with the results reported by Enache et al. (2005) on the heat resistance of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* in white grape juice concentrate. *E. coli* O157:H7 was found to have the highest heat resistance for all temperatures tested followed by *L. monocytogenes* and *S. enterica*.

Results of this study are also similar to those reported by Gabriel and Arellano (2014) where *E. coli* O157:H7 cocktail exhibited the greatest resistance among the test pathogens in heat-treated young coconut liquid endosperm with a D_{55} of 23.20 min. Another study of Gabriel (2014) showed that increasing the heating temperatures also decreased the D_T values of *E. coli* O157:H7 in heated apple juice. The significant effects of heating temperatures and exposure times on microbial inactivation in food systems were similarly discussed by Gabriel and Arellano (2014), Gabriel and Ubana (2006), and Murphy et al. (2004). Variations in the reported thermal resistance of the test pathogens in food may also be due to the intrinsic properties of the suspending food systems (Jay et al., 2005; Li Wan Po et al., 2002). Vertigo (2003) reported a lower D_T values of acid-adapted *E. coli* in the more acidic apple juice.

The variations in thermal resistance of pathogens due to physicochemical properties of suspending media have also been highlighted in the works reported by Murphy et al. (2004) and Sharma et al. (2005), where *L. monocytogenes* were found significantly more heat resistant than *E. coli* O157:H7 and *Salmonella enterica* when suspended in low acid foods such as ground pork and cantaloupe juice. In fruit systems, Li Wan Po et al. (2002) showed that pH, TA (titrable acidity) and SS (soluble solid) significantly affect the thermal inactivation rates of microorganisms. Also, the inherent differences in the thermal resistance

Table 1
Inactivation kinetic parameters of test pathogens in heat- and UV-C-treated coconut liquid endosperm.

Organisms	Thermal (D_T and z) and UV-C (D_{UV-C}) inactivation parameters[1]					
	D_T values[2] (min) per heating temperature				z values[3] (°C)	D_{UV-C}[4] (mJ/cm^{2})
	D_{55}	D_{57}	D_{60}	D_{63}		
E. coli O157:H7	19.75 ± 2.26^{a,w}	10.79 ± 1.89^{a,x}	3.38 ± 0.62^{a,y}	0.46 ± 0.04^{a,z}	4.95 ± 0.12^{b}	25.26 ± 4.36^{a}
S. enterica	3.08 ± 0.51^{c,w}	2.60 ± 0.68^{c,w}	0.89 ± 0.06^{c,x}	0.25 ± 0.01^{b,x}	7.10 ± 0.15^{a}	17.30 ± 1.59^{b}
L. monocytogenes	11.68 ± 3.51^{b,w}	4.53 ± 0.29^{b,x}	1.83 ± 0.40^{b,y}	0.26 ± 0.01^{b,y}	5.12 ± 0.43^{b}	24.65 ± 5.23^{ab}

[a,b,c]Inactivation kinetic parameters on the same column followed by the same letters are not significantly different ($P > 0.05$).
[w,x,y,z]Inactivation kinetic parameters on the same row followed by the same letters are not significantly different ($P > 0.05$).
[1] Inactivation kinetic parameters are presented as averages of 4 values ± standard deviation obtained from 2 independent experimental runs.
[2] D_T value is the length of time (min) of heating to temperature T that will result in 90% (1 log unit) reduction in the microbial population.
[3] z value is the increase in heating temperature that will reduce the D_T value of a pathogen by 90% (1 log unit).
[4] D_{UV-C} is the UV-C energy dose that will result in 90% (1 log unit) reduction in the microbial population at the tested irradiance value of 0.42 mW/cm^{2}.

characteristics between species may be some of the factors that can explain the disparities between reported D_T values. Other studies have established the influences of other factors on the thermal inactivation, which included suspending medium composition, microbial growth temperature, initial reference strain population and culture age (Abe et al., 2004; Juneja et al., 2001; Mazzotta, 2001; Mulak et al., 1995; Sharma et al., 2005; Smith et al., 2001). Variations in the physiological states of the challenge organisms have also been reported to significantly affect the resistance towards thermal treatment. Chen et al. (2013) found that desiccated *Salmonella* cells were more heat-resistant as compared to cells not exposed to such stress. Gabriel (2012, 2013) and Gabriel and Arellano (2014) also reported the significant effects of stresses on the thermal resistance of different pathogens in fruit juices.

The z-values of all test organisms were also determined. The z value refers to the increase in heating temperature (°C) necessary for the thermal death time curve (Fig. 3) to traverse 1-log unit cycle (10-fold reduction in the D value). The z value of the tested cocktail of *E. coli* O157:H7 determined in this study is close to that reported by Vertigo (2003) for acid-adapted *E. coli* O157:H7 (6.0 °C). Stringer et al. (2000) also reported a z value of 7.4 °C for *E. coli* O157:H7 in apple juice. Additionally, Gabriel et al. (2014) reported a z value of 8.9 ± 0.1 °C for acid-adapted *E. coli* O157:H7 in an apple juice medium. The higher z values from these previous studies can be attributed to the acid adaptation of *E. coli* O157:H7 prior to heat inactivation. According to Archer (1996) and Bearson et al. (1997) exposures to sublethal stresses such as an acidic environment allow microorganisms to adapt and develop cross-protective mechanisms against other stresses like thermal treatment through gene responses and adaptive mutations. Moreover, the z value of *S. enterica* cocktail was also close to the z value obtained in the study of Enache et al. (2005) but not in the case of *E. coli* O157:H7 and *L. monocytogenes*. In their study, the z values reported for the test pathogens on stationary phase condition in white grape juice concentrate were 8.8 ± 0.3 °C for *S. enterica*, 8.0 ± 0.7 °C for *L. monocytogenes*, and 9.2 ± 0.4 °C for *E. coli* O157:H7. Disparities in the previously reported z values and those obtained from this study may also be attributed to inter and intra- species variations in the heat resistance within and among the treated pathogens.

3.2. $D_{UV\text{-}C}$ values of test pathogens in irradiated coconut liquid endosperm

Results of the UV-C inactivation studies showed that all tested cocktails of organisms also exhibited logarithmic linear inactivation behavior as shown in the inactivation curves in Fig. 4. This first-order inactivation kinetics of the inactivation curves may suggest that all test organisms in the bacterial cocktails had similar resistance to the UV-C, or that cellular injury and death simultaneously take place (Peleg, 2006). Table 1 also shows $D_{UV\text{-}C}$ values of the test organisms in the irradiated treated coconut liquid endosperm. These inactivation kinetic parameters are measures of the UV-C energy (mJ/cm^2) dose necessary for the population of the organism suspended in the coconut liquid endosperm to be reduced by 90%.

Results revealed that among the test organisms, the cocktail of *E. coli* O157:H7 exhibited the greatest resistance towards UV-C with a $D_{UV\text{-}C}$ value of 25.26 ± 4.36 mJ/cm^2. This was followed by the cocktail of *L. monocytogenes* with $D_{UV\text{-}C}$ value of 24.65 ± 5.23 mJ/cm^2. The *S. enterica* cocktail was the least resistant with $D_{UV\text{-}C}$ value of 17.30 ± 1.59 mJ/cm^2. Despite these differences in the computed $D_{UV\text{-}C}$ values, results of the study revealed that the UV-C resistance of *L. monocytogenes* cocktail was not significantly different (P > 0.05) to that of *E. coli* O157:H7 and *S. enterica* cocktails. However, the resistance of *E. coli* O157:H7 cocktail to UV-C treatment was significantly different from the tested cocktail of *S. enterica*. These variations in the D values of the organisms can be attributed to the inherent characteristic of the suspending medium and the implicit factor or innate resistance of the test species (Gabriel, 2015b). Nevertheless, considering the $D_{UV\text{-}C}$ values for all test organisms an appropriate target reference organisms should be the tested cocktail of *E. coli* O157:H7 since the UV-C resistance was higher than that of the other two test organisms.

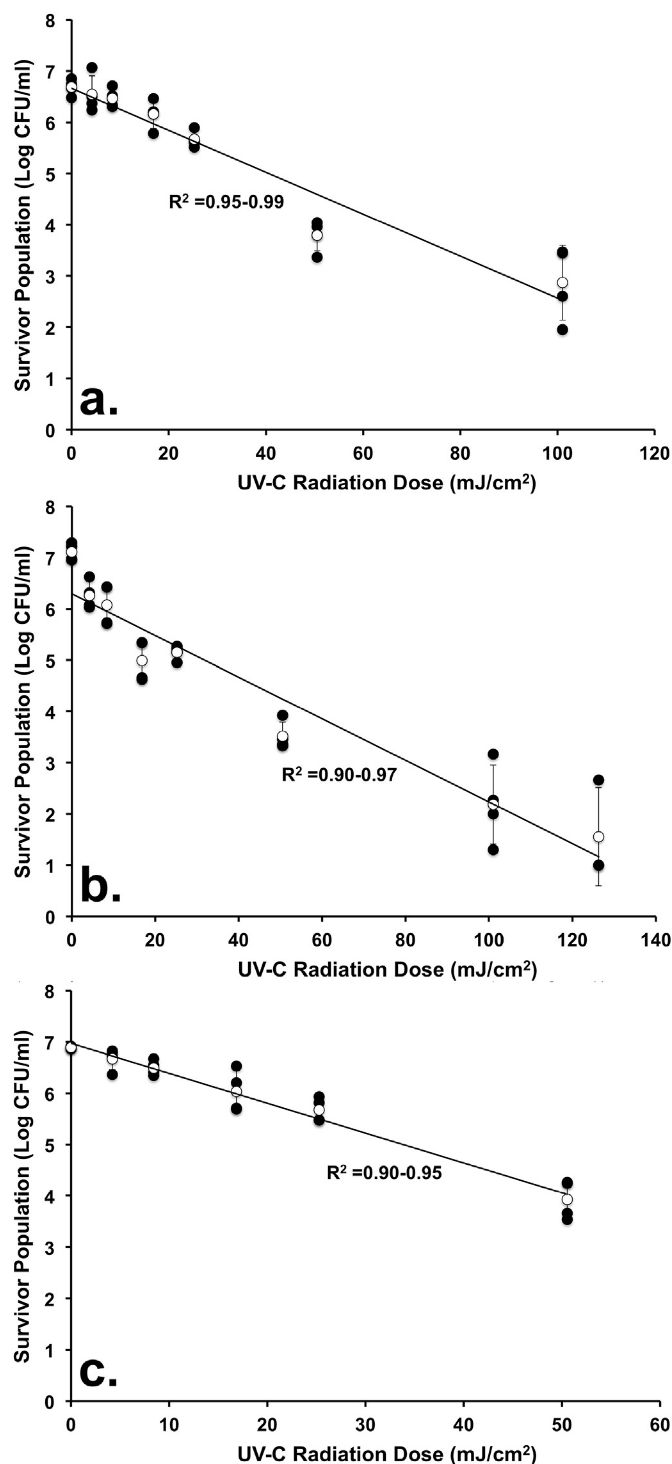

Fig. 4. UV-C inactivation curves of (a) *E. coli* O157:H7, (b) *S. enterica*, and (c) *L. monocytogenes*. The $D_{UV\text{-}C}$ values were determined from these curves. Black markers represent values obtained from internal and external replications; white markers and error bars represent average values and standard deviations calculated from four data points, respectively. The R^2 ranges represent the coefficients of determination obtained from the 4 replications. External replications represent independent experiment runs. Internal replications refer to duplicate analyses per experiment run.

The results of this study are similar to those reported by Gabriel and Colambo (2016) for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* in

UV-C treated coconut liquid endosperm with different physicochemical characteristics. In their study, *E. coli* O157:H7 was also found to have greater UV-C resistance, followed by *S. enterica*, and *L. monocytogenes*. On the other hand, Gabriel (2015b) reported an opposite trend in D_{UV-C} values in non-stirred coconut liquid endosperm. Furthermore, the D_{UV-C} values reported from previous studies were lower compared to the D_{UV-C} values obtained from this study. Similar with D_T values, differences in the reported D_{UV-C} values can be explained by the variations of the product physicochemical characteristics and experimental set up employed.

The observed efficacies of UV-C against all test pathogens are in agreement with previously reported studies that concluded that UV-C radiation can be considered as a promising alternative to traditional heat treatments, together with its other advantages such as absence of toxic residues, the simple operation and maintenance and the low energy consumption (Guerrero-Beltrán and Barbosa-Cánovas, 2004). However, the recommended 5D reduction (99.999%) in the reference organism population imposed by the USDA may not be easily achieved because of the dependence of UV-C efficacy on the optical properties of the treated liquid (e.g., turbidity, absorption coefficient) (Koutchma, 2008; Murakami et al., 2006). This gap may be addressed by using UV-C as one of biocidal processes in hurdle technology-based process for products such as coconut liquid endosperm beverages. This was explored in combining UV-C irradiation with mild heat treatment at 55 °C.

3.3. Lethal rates of combined heat- and UV-C against test pathogens in coconut liquid endosperm

In this study, only 3D (99.9%) heating processes at 55 °C were calculated for the test organisms with the hypothesis that this could further be enhanced by the simultaneous exposure to UV-C radiation, to achieve the recommended 5D (99.999%) process. This hypothesis was tested by exposing all organisms to their respective calculated 3D heating process (59 min, 9 min and 35 min for *E. coli* O157:H7, *S. enterica* serovars and *L. monocytogenes*, respectively) and subjecting them to simultaneous UV-C irradiation. The overall lethal rates (heat + UV-C) were compared with lethality due to heat to assess the contribution UV-C made to microbial reduction. Fig. 5 simultaneously illustrates the heat penetration profile and the corresponding heat lethality against the test organisms in the heated coconut liquid endosperm. The total lethality (F_{55} value) was calculated using the Simpson's Rule (Gabriel, 2014; Da- Wen Sun, 2012). The integrated lethal rate (*F* values) that included lethal rates during come-up time and cool down were calculated and depicted in the shaded area under the curve and are also summarized in Table 2.

In the 3D process schedule at 55 °C with UV-C treatment of the coconut liquid endosperm sample where the test organisms were inoculated, a total of 5.94, 5.62 and 6.20 logs were reduced from the initial population of approximately 8 log CFU of separate cocktails of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*. The fraction of the total lethality due to heating was calculated to be equal to 4.12, 5.46 and 4.18 log reduction for the cocktails of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* respectively. The > 3 log reductions in all test organisms are due to inactivation during come up and cool down, which can be controlled and accounted for in succeeding process designs. The observed % inactivation due to heat account for 69.3, 97.2, and 67.4% of the reduction in the cocktail of *E. coli* O157:H7, *S. enterica* and *L. monocytogenes*, respectively. On the other hand, the total lethality due to UV-C irradiation was calculated to be equal to 1.82 (30.7%), 0.16 (2.8%) and 2.02 (32.6%) log reduction for the cocktail of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, respectively. Hence, in all combined heating-UV-C processes, heating at 55 °C accounted for the significant fraction of inactivated population. Moreover, these results showed that UV-C may simultaneously be employed with mild heat (55 °C) to further increase lethality and achieve the 5D process against the target organisms.

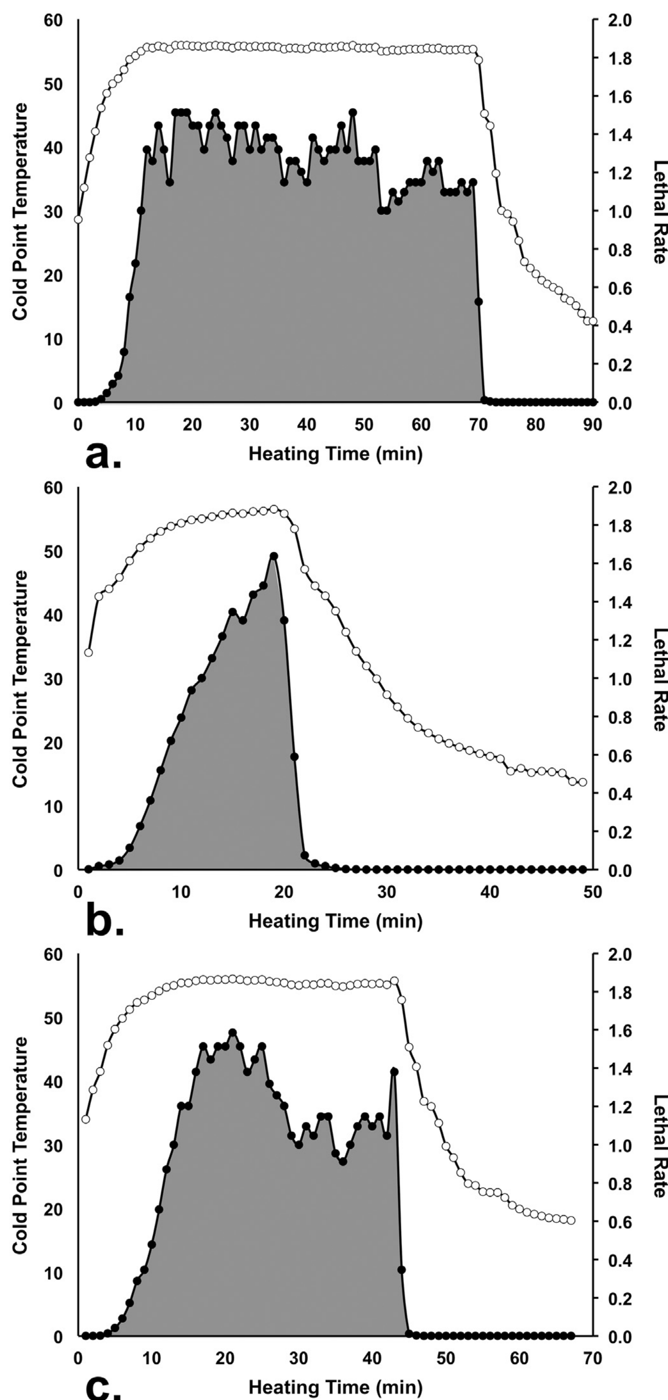

Fig. 5. Temperature profile (white markers) and corresponding heat lethal rates (black markers) plots for (a) *E. coli* O157:H7, (b) *S. enterica*, and (c) *L. monocytogenes* in young coconut endosperm subjected to simultaneous mild heating (55 °C) and UV-C irradiation. The shaded areas under the curve represent total microbial inactivation due to heat alone.

The combination of UV-C radiation and mild heat (UV + H) has demonstrated a synergistic inactivation of *E. coli* in fruit juices (Gayán et al., 2012a). In particular, a combined UV + H treatment (3.9 J/mL at 55 °C for 3.6 min) has been designed capable of inactivating 5 logs of a cocktail of pathogenic *E. coli* strains in apple juice without impairing the quality attributes of the product (Gayán et al., 2012b). Similarly, Gouma et al. (2015) reported that inactivation of *Escherichia coli* in commercial apple using combinations of UV-C, heat (55 °C) and dimethyl dicarbonate (DMDC) resulted in a synergistic lethal effect,

Table 2
Individual and combined lethal rates of heat (55 °C)- and UV-C treatments against test pathogens in coconut liquid endosperm.

Parameters	*E. coli* O157:H7	*S. enterica*	*L. monocytogenes*
Total log reduction[1]	5.94	5.62	6.20
F_{55} value (min)[2]	81.31	16.83	48.84
D_{55} value (min)	19.75	3.08	11.68
Log reduction due to heat (55 °C)[3]	4.12	5.46	4.18
Log reduction due to UV-C[4]	1.82	0.16	2.02

[1] Calculated from the difference between inoculated population and the survivor population after combined treatments.

[2] Integrated lethal rates due to heating at 55 °C calculated from start of heating to the end of cool down.

[3] Calculated by dividing the F_{55} value with the D_{55} value per test organism.

[4] Calculated from the difference between the total log reduction and the log reduction due to heat.

reducing the total treatment time and UV dose, with the synergistic lethal effect being higher when larger concentrations of DMDC were added to the apple juice.

The efficacy of the combined processes of UV light and mild temperatures for the inactivation of *Salmonella enterica* was also investigated by Gayán et al. (2012c). Results of their study revealed an inactivating synergistic effect when UV light and heat treatment was applied simultaneously. However, a less synergistic effect was observed by applying UV light first and then followed by heat. They also reported that the pH and water activity of the treatment medium did not change the UV tolerance. On the other hand, UV-tolerance decreased exponentially by increasing the absorption coefficient of the suspending medium.

The use of mild thermal process and other non-thermal technologies in the inactivation of foodborne bacteria deserves further investigation especially when applied singly or in combination. These results are useful in the establishment of process schedules for the manufacture of safe finished products. Similar studies should be conducted to investigate the inactivation of spoilage-causing organisms for shelf life extension. Quality evaluation should also be done to further evaluate the utility of the technologies under investigation.

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References

Abe, K., Saito, N., Kasuga, F., Yamamoto, S., 2004. Prolonged incubation period of salmonellosis associated with low bacterial doses. J. Food Prot. 67, 2735–2740.
Alabastro, E.F., 1987. Establishment of Thermal Processes for Food Products. University of the Philippines Diliman, Manila.
AOAC, 2007. Official methods of analysis of AOAC international, 18th ed. Association of Official Analytical Chemists, Washington.
Archer, D.L., 1996. Preservation microbiology and safety: evidence that stress enhances virulence and triggers adaptive mutations. Trends Food Sci. Technol. 7, 91–95.
Alave, K.L., 2011. Coconut water exports jump 315%. Philippine Daily Inquirer, 17 October 2011. http://business.inquirer.net/25199/coconut-water-exports-jump-315.
Bearson, S., Bearson, B., Foster, J.W., 1997. Acid stress responses in enterobacteria. FEMS Microbiol. Lett. 147, 173–180.
Bell, W., Rouseff, R., 2004. Examination of volatiles in Florida grapefruit juice when heated on different contact surfaces. In: Fifty-Fifth Citrus Processor's Meeting, Lake Alfred, FL. October 21, 2004, (Abstract).
Bureau of Product Standards, 2006. Philippine national standard for chilled young coconut water/drink-specification. PNS/BAFPS 28:2006. ICS 67.160.20. http://spsissuances.da.gov.ph/attachments/article/1094/PNS-BAFS28-2006-BukoJuice.pdf.
Butz, P., Tauscher, B., 2002. Emerging technologies: chemical aspects. Food Res. Int. 35, 279–284.
Chen, Z., Diao, J., Dharmasena, M., Ionita, C., Jiang, X., Rieck, J., 2013. Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter. Appl. Environ. Microbiol. 79, 7013–7020.
Damar, S., 2006. Processing of Coconut Liquid Endosperm With High Pressure Carbon Dioxide Technology (Unpublished Thesis). University of Florida. http://ufdcimages.uflib.ufl.edu/UF/E0/01/55/41/00001/damar_s.pdf.
De Roeck, A., Mols, J., Duvetter, T., Van Loey, A., Hendrickx, M., 2010. Carrot texture degradation kinetics and pectin changes during thermal versus high-pressure/high-temperature processing: a comparative study. Food Chem. 120, 1104–1112.
Enache, E., Chen, Y., Awuah, G., Economides, A., Scott, V., 2005. Resistance Parameters for Pathogens in White Grape Juice Concentrate. Center for Technical and Laboratory Services, Food Products Association, 1350 I Street N.W., Suite 300, Washington, D.C. 20005, USA. MS 05-247.
Federal Register, 2001. Final rule. Hazard analysis and critical control points (HACCP): procedures for the safe and sanitary processing and importing of juice. vol. 66. Federal Register, U.S. Food and Drug Administration, pp. 6138e6202.
Food and Agricultural Organization (FAO) AG21: magazine. November, 2000. New sports drink: coconut liquid endosperm. http://www.fao.org/ag/magazine/9810/spot3.htm.
Forbes, E.G., 2013. Outlook for the Coconut Industry. Bangko Sentral ng Pilipinas. Philippine Coconut Authority, Manila, Philippines.
Gabriel, A.A., 2012. Inactivation of *E. coli* O157:H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. Food Control 23, 425–432.
Gabriel, A.A., 2013. Influences of simultaneous physicochemical stresses on injury and subsequent heat and acid resistances of *Salmonella* Enteritidis in apple juice. Food Control 31, 28–34.
Gabriel, A.A., 2014. Precision Food Processing: Addressing Consumer Demands for Safety and Quality through Predictive Model Building. SEARCA Agriculture & Development Discussion Paper Series. No. 2014-4.
Gabriel, A.A., 2015a. Combinations of selected physical and chemical hurdles to inactivate *Escherichia coli* O157:H7 in apple and orange juices. Food Control 50, 722–728.
Gabriel, A.A., 2015b. Previous physicochemical stress exposures influence subsequent resistance of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* to ultraviolet-C in coconut liquid endosperm beverage. Int. J. Food Microbiol. 201, 7–16.
Gabriel, A.A., Arellano, R.U., 2014. Decimal reduction times of acid-adapted and non-adapted *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in young *Cocos nucifera* Linn. liquid endosperm. Food Control 37, 21–26.
Gabriel, A.A., Colambo, J.C.R., 2016. Comparative resistance of selected spoilage and pathogenic bacteria in ultraviolet-C-treated, turbulent-flowing young coconut liquid endosperm. Food Control 69, 134–140.
Gabriel, A.A., Estilo, E.C., 2015. Influences of malic acid and nisin supplementations on the decimal reduction times of Escherichia coli O157:H7 in mildly-heated young coconut liquid endosperm. Food Control 50, 645–651.
Gabriel, A.A., Nakano, H., 2010. Responses of *E. coli* O157:H7, *L. monocytogenes* 1/2 c and *Salmonella enteritidis* to pH, aw and temperature stress combinations. Food Control 21, 644–650.
Gabriel, A.A., Ubana, M.A., 2006. Decimal reduction times of *Salmonella typhimurium* in *guinataang kuhol*: an indigenous Filipino dish. Lebensm-Wiss. Technol. 40, 1108–1111.
Gabriel, A.A., Aguila, M.L., Tupe, K.A., 2015. Application of ultraviolet-C radiation to inactivate acid-and-desiccation stressed *Salmonella enterica* in young and mature coconut liquid endosperm mix beverage. Food Control 51, 425–432.
Gabriel, A.A., Aba, R.P.M., Tayamora, D.J.L., Colambo, J.C.R., Siringan, M.A.T., Rosario, L.M.D., Tumlos, R.B., Ramos, H.J., 2016. Reference organism selection for microwave atmospheric pressure plasma jet treatment of young coconut liquid endosperm. Food Control 69, 74–82.
Gayán, E., Serrano, M., Monfort, S., Álvarez, I., Condón, S., 2012a. Pasteurization of apple juice contaminated with Escherichia coli by a combined UV–mild temperature treatment. Food Bioprocess Technol. 6, 3006–3016.
Gayán, E., Serrano, M.J., Monfort, S., Álvarez, I., Condón, S., 2012b. Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice. J. Food Eng. 113, 598–605.
Gayán, E., Serrano, M.J., Raso, J., Alvarez, I., Condon, S., 2012c. Inactivation of *Salmonella enterica* by UV-C light alone and in combination with mild temperatures. Appl. Environ. Microbiol. 78, 8353–8361.
Gouma, M., Gayán, E., Raso, J., Condón, S., Álvarez, I., 2015. Influence of dimethyl dicarbonate on the resistance of *Escherichia coli* to a combined UV-heat treatment in apple juice. Front. Food Microbiol. 6, 501.
Guerrero-Beltrán, J.A., Barbosa-Cánovas, G.V., 2004. Advantages and limitations on processing foods by UV light. Food Sci. Technol. Int. 10, 137–147.
Guerrero-Beltran, J.A., Barbosa-Canovas, G.V., 2005. Reduction of Saccharomyces cerevisiae, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. J. Food Process Eng. 28, 437–452.
Jay, J.M., Loessner, M.J., Golden, D.A., 2005. Food protection with high temperature. In: Modern Food Microbiology. Springer Science + Business, New York, USA (Ch. 17).
Juneja, V.K., Eblen, B.S., Ransom, G.M., 2001. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken: determination of D- and Z-values. J. Food Sci. 66, 146–152.
Kathariou, S., 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. J. Food Prot. (11), 1687–1829.
Koutchma, T., 2008. UV light for processing foods. Ozone Sci. Eng. 30, 93–98.

Koutchma, T., 2014. Preservation and Shelf Life Extension: UV Applications for Fluid Foods. Academic Press, London, pp. 1–61.
Koutchma, T., Parisi, B., Patazca, E., 2007. Validation of a UV coiled tube reactor for fresh fruit juices. J. Environ. Eng. Sci. 6, 319–328.
Leistner, L., Gould, G., 2002. Hurdle Technologies- Combinations of Treatments for Stability, Safety and Quality. Kluwer Academic Publishers, New York, pp. 1e15.
Leite, C.C., Santana, L.R.R., Sant'Ana, M.E.B., Assis, P.N., 1998. Avaliação microbiológica da água de coco produzida e comercializada na cidade de Salvador e BA. In: In 16st Congresso Brasileiro de Ciência e Tecnologia de Alimentos, Anais. Sociedade Brasileira de Ciência e Tecnologia de Alimentos, Rio de Janeiro, Brazil.
Lopez-Malo, A., Palou, E., 2002. Ultraviolet light and food preservation. In: Barbosa-Cánovas, G., Tapia, M.S., Cano, P. (Eds.), Novel Food Processing Technologies. CRC Press, Boca Raton, USA, pp. 405–422.
Li Wan Po, J.M., Piyasena, P., Mckellar, R.C., Bartlett, F.M., Mittal, G.S., Lu, X., 2002. Influence of simulated apple cider composition on the heat resistance of *Escherichia coli* O157:H7. Lebensm-Wiss. Technol. 35, 295–304.
Mak, P.P., Ingham, B.H., Ingham, S.C., 2001. Validation of Apple Cider Pasteurization Treatments against *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. J. Food Prot. 64 (11), 1653–1877.
Mazzotta, A.S., 2001. Thermal inactivation of stationary-phase and acid-adapted *E. coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. J. Food Prot. 64, 315–320.
Melo, N.P.M., Cardonha, A.M.S., Oliveira, A.C.F., 2003. Qualidade microbiológica das águas de coco envasadas e comercializadas na cidade de Natal e RN. Hig. Aliment. 17, 113–114.
Moats, W.A., 1971. Kinetics of thermal death of bacteria. J. Bacteriol. 105, 165–171.
Mulak, V., Tailliez, R., Eb, P., Becel, P., 1995. Heat resistance of bacteria isolated from preparations based on seafood products. J. Food Prot. 58, 49–53.
Murakami, E.G., Jackson, L., Madsen, K., Schickedanz, B., 2006. Factors affecting the ultraviolet inactivation of *Escherichia coli* K12 in apple juice and a model system. J. Food Process Eng. 29, 53–71.
Murphy, R.Y., Duncan, L.K., Beard, B.I., Driscoll, K.H., 2004. D and z values of *Salmonella*, *Listeria innocua* and *Listeria monocytogenes* in fully cooked poultry products. J. Food Sci. 68, 1443–1447.
Patil, S., Bourke, P., Frias, J.M., Tiwari, B.K., Cullen, P.J., 2009. Inactivation of *Escherichia coli* in orange juice using ozone. Innovative Food Sci. Emerg. Technol. 10, 551–557.
Peleg, M., 2006. Isothermal microbial heat inactivation. In: Advanced Quantitative Microbiology for Foods and Biosystems, Models for Predicting Growth and Inactivation. CRC Press, Taylor & Francis Group, Boca Raton, Fla, pp. 1–48.
Reddy, K.V., 1995. Processing of green coconut water for bottling (Masters in Technology Thesis) In: Diary and Food Engineering. Department of Agricultural Engineering, Indian Institute of Technology, Kharagpur, India.
Reddy, M.B., Love, M., 1999. The impact of food processing on the nutritional quality of vitamins and minerals. Adv. Exp. Med. Biol. 459, 99–106.
Rolle, R., 2007. Good practice for the small-scale production of bottled coconut liquid endosperm. In: Agricultural and Food Engineering Training and Resource Materials. Food and Agriculture Organization of the United Nations, Rome, Italy.
Sastry, S.K., Datta, A.K., Worobo, R.W., 2008. Ultraviolet light. J. Food Sci. Suppl. 90–92.
Sharma, M., Adler, B.B., Harrison, M.D., Beuchat, L.R., 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. Lett. Appl. Microbiol. 41, 448–453.
Shaw, P.E., 1982. The flavor of non-alcoholic fruit beverages. In: Morton, I.D., Macleod, A.J. (Eds.), Food Flavours, Part B: The Flavor of Beverages. Elsevier Science Pub., New York, pp. 337–367.
Salleh-Mack, S.Z., Roberts, J.S., 2007. Ultrasound pasteurization: the effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. Ultrason. Sonochem. 14, 323–329.
Shreirer, P., Drawert, F., Junker, A., Mick, W., 1977. The quantitative composition of natural and technologically changed aromas of plants. II. Aroma compounds in oranges and their changes during juice processing. Z. Lebensm. Unters. Forsch. 164, 188–193.
Smith, S.E., Maurer, J.L., Orta-Ramirez, A., Ryser, E.T., Smith, D.M., 2001. Thermal inactivation of *Salmonella spp*., *Salmonella typhimurium* DT104 and *Escherichia coli* O157:H7 in ground beef. J. Food Sci. 66, 1164–1168.
Stringer, S.C., George, S.M., Peck, M.W., 2000. Thermal inactivation of *Escherichia coli* O157:H7. J. Appl. Microbiol. Symp. Suppl. 88, 79S–89S.
Sun, Da-Wen, 2012. Thermal Food Processing New Technologies and Quality Issues, Second edition. CRS Press, Taylor & Francis Group, NW, USA.
United Nations Conference on Trade and Development (UNCTAD), 2012. Coconut. Retrieved February 15, 2015, from United Nations Conference on Trade and Development. http://www.unctad.info/en/Infocomm/AACPProducts/COMMODITY-PROFILE—Coconut2/.
United States Department of Agriculture (USDA) and United States Department of Health and Human Services (USDHHS), 2001. Hazard Analysis and Critical Control Point (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice; Final Rule, 21 CFR Part 120, 66 FR 6137–6202, 19 January 2001. U.S. Food and Drug Administration, Washington D.C.
US-FDA (United States Food and Drug Administration), 2002. Ultraviolet radiation for the processing and treatment of food. In: Code of Federal Regulations. 21 (Part 179.39).
Vertigo, M., 2003. Thermal Inactivation Kinetics of *Escherichia coli* and *Alicyclobacillus acidoterrestris* in Orange Juice. University of Florida. http://www.mtifoodscience.com/docs/moody_v.pdf.
Vojdani, J.D., Beuchat, L.R., Tauxe, R.V., 2008. Juice-associated outbreaks of human illness in the United States, 1995 through 2005. J. Food Prot. 71, 356–364.
Walter, E.H.M., Kabuki, D.Y., Esper, L.M.R., Sant'Ana, A.S., Kuaye, A.Y., 2009. Modelling the growth of *Listeria monocytogenes* in fresh green coconut (*Cocos nucifera* L.) water. Food Microbiol. 26, 653–657.
Yong, J.W.H., Ge, L., Ng, Y.F., Tan, S.N., 2009. The chemical composition and biological properties of coconut (*Cocos nucifera L*.) water. Molecules 14, 5144–5164.