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Thermal and ultraviolet-c inactivation of *Salmonella enterica* in cold-pressed virgin coconut oil

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ABSTRACT

Virgin Coconut Oil (VCO) consumption as functional food has recently become popular together with increasing evidence of its health benefits. However, cold-pressed VCO types are highly manually produced without the application of kill step to ensure safety from possible pathogenic contaminants. The study therefore determined the inactivation behavior and kinetic parameters of *Salmonella enterica* in VCO subjected to mild heat and ultraviolet-c (UV–C) irradiation. When exposed to 50–70 °C, *S. enterica* exhibited a biphasic inactivation behavior with initial, logarithmic linear population decline of 2.07–2.39 log CFU/mL. An inactivation tail followed where minimal population changes took place. Total inactivated cells ranged from 2.28 to 2.35 log CFU/mL, which were achieved from 2. o to 100 min. Similar behavior was observed in UV-C-irradiated VCO at surface irradiance of 1.25–3.99 mW/cm^2. Population decline in the log-linear inactivation phase ranged from 2.59 to 4.00 log CFU/mL, achieved after 0.33–1.20 min. After irradiation for 2.0 min, the total inactivated population ranged from 2.68 to 4.17 log CFU/mL. Complete elimination of *S. enterica* was not observed in any of the highly contaminated VCOs. These results provide baseline information for the establishment of pasteurization processes to achieve VCO safety.

1. Introduction

Saturated fatty acids have been linked with increased risk of cardiovascular diseases (CVDs) (Piepoli et al., 2016). Such fatty acid type is found in coconut oil, albeit medium-chained, and exhibits good digestibility (Che Man & Marina, 2006). The Philippine National Standards/Bureau of Agriculture and Fisheries Product Standards (PNS/BAFPS 22:2007) published by the Bureau of Product Standards of the Department of Trade and Industry (BPS-DTI, 2007) specifically pointed out that the saturated fatty acids in virgin coconut oil (VCO) are distinct from those derived from animals, which are mainly consisted of long-chain fatty acids. The study conducted by Kaunitz and Dayrit (1992) established that dietary coconut oil did not lead to increased mortality and morbidity related to CVDs. This was confirmed by a more recent study conducted by Chinwong, Chinwong, and Mangklabruks (2017), which showed that daily consumption of VCO increased the High-Density Lipoproteins in healthy participants. Unlike the refined, bleached, and deodorized (RBD) coconut oil that is produced by employing high temperatures ranging from 204 to 245 °C (O'Brien, 2004), VCO is obtained from fresh, mature, coconut drupes by mechanical or natural means, with or without the use of heat, and without the chemical refinement (Villarino, Dy, & Lizada, 2007). Marina, Che Man and Amin (2009) explained that since its first introduction, VCO consumption has increased, particularly in South East Asia where it is consumed as functional food, contrary with RBD oils, which are produced mainly for cooking.

The Implementing Rules and Regulations to Enforce Standards in the Production and Marketing of VCO of the Philippine Coconut Authority states that processors shall declare in the product label the exact process with which the VCO was produced, i.e., traditional or *latik* process with heating, fermentation with heat, fermentation without heat, centrifuge process, or expelling process with or without cooling system (DA-PCA, 2005). In the process flow detailed by Srivastava, Semwal, and Sharma (2018) for cold-extracted VCO (CEVCO), milk was extracted from grated coconut meat, which was there after placed in a tank for 20–24 h at 35–40 °C and 75% relative humidity. Water and cream were thereafter removed prior to filtration of oil. This particularly long process at ambient conditions that permits microbial survival and possible proliferation, and the absence of a kill step prior to packaging and distribution of CEVCO or cold-pressed VCO makes the

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commodity a probable vector of foodborne illnesses. In fact coconut products that are intermediate ingredients in the production VCO have been associated with foodborne illnesses, such as cholera and salmonellosis due to contaminated meat and the milk extracted from the pressed meat (CDC, 1991; Schaffner, Mosbach, Bibit, & Watson, 1967; Taylor et al., 1993; USFDA, 2018). Hence, introduction of pathogenic microorganisms to these ingredients could eventually lead to the contamination of the finished product, which could become vectors of foodborne illnesses.

Food decontamination from pathogenic and spoilage microorganisms with heating is still considered the more traditional but effective means of food processing (Mak, Ingham, & Ingham, 2001) particularly in developing countries were VCOs are produced. However, with the increase in consumer demand for fresh, high quality and nutritious food products, heat treatment application may limit product marketability of VCO, since exposure to heating may significantly reduce physical, nutritive and sensory attributes (Gabriel, 2014). Alternative, nonthermal processes such as the use of ultraviolet-C (UV–C) irradiation to decontaminate food products may also be explored (Koutchma, 2014, pp. 1–61). UV-C technology is a non-thermal process harnessing the germicidal ultraviolet light region of 200–800 nm (Gayán, Condón, & Álvarez, 2014). The use of UV-C for treatment for coconut products involving young coconut liquid endosperm, coconut milk and desiccated coconut meat flakes has been demonstrated by earlier studies (Gabriel, 2015; Gabriel, Aguila, & Tupe, 2015; Gabriel & Colambo, 2016; Gabriel, Tongco, & Barnes, 2017). Cold-pressed VCO is a particularly vulnerable commodity because of the lack of kill step applied throughout its production process flow. Further, application of a kill step on the finished product rather than the raw material or intermediate ingredient provides better margin of safety to the commodity. Thus, this study was conducted to determine the efficacy of heat- and UV-C irradiation as anti-*Salmonella* treatments for cold-pressed VCO. The inactivation behavior and kinetic parameters were determined, which can be used in the establishment of post extraction kill step against the tested organism.

2. Methodology

2.1. Cold-pressed VCO

A commercially available cold-pressed, centrifuged virgin coconut oil (VCO) was procured for this study. The samples in 250 mL bottles were produced by Carica Herbal Health Products, Inc. (Manila, Philippines). The products were declared as 'cold-pressed, centrifuged.' Samples from the same brand and same production lot were chosen to minimize differences in composition and physicochemical properties, which could potentially affect the results of the study. The VCO samples were stored inside a 35 °C incubator for 24 h prior to use to prevent solidification. For pH determination, the method of US EPA Method 9045D (Horiba, 2015) was adapted. Twenty milliliters of water was mixed with equal amount of VCO in a 100 mL beaker (Pyrex USA). The mixture was covered and stirred using a magnetic spinner (Corning, PC-420D, Mexico) at 1150 rpm for 5 min. After stirring, the solution was let to stand for 15 min to allow the sample and water to separate. The pH of the aqueous phase was measured and recorded. Measurement was done in 3 trials, and the average as well as standard deviation of the readings were determined. Free Fatty Acid was determined following the AOAC Official Method 940.28 for Fatty Acids (Free) in Crude and Refined Oils. In 250 mL Erlenmeyer flask, 7.05 g of well-mixed VCO was weighed, then 50 mL of neutralized 95% ethyl alcohol (RTC Laboratory, Philippines) was added. The mixture was thereafter titrated with 0.25 N NaOH (RCI Labscan, Thailand) until permanent faint pink appears and persist for 1 min. The obtained data was reported percent free fatty acids expressed in oleic acid with the used amount of 0.25 N NaOH in titration corresponding to the percentage. Finally, the UV-C absorbance/transmittance of the VCO sample was also determined at 254 nm at 1 cm path length using a UV/Vis double beam spectrophotometer (Shimadzu UV-VIS Spectrophotometer, Model UV-1900, Tokyo, Japan). The background microflora of the VCO, namely Total Plate Count and Yeast and Molds Count were also checked by surface plating appropriate dilutions on Plate Count Agar (PCA, HiMedia, Mumbai, India) and acidified Potato Dextrose Agar (PDA, HiMedia), respectively. Microbial populations were determined after 24 h and 120 h on PCA and PDA, respectively.

Table 1
Heat and UV-C treatment protocols employed in the VCO inactivation studies.

Inactivating Agent	Treatment Times (min)
Heating Temperature (°C)	
50	0, 10, 15, 20, 30, 40, 60, 80, 100
55	0, 5, 15, 20, 30, 40, 60, 80, 100
60	0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 5.0, 20
65	0, 0.25, 0.5, 1.0, 3.0, 5.0, 10, 15
70	0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 0.75, 1.0, 2.0
UV-C Irradiance (mW/cm^2)	
1.25	0, 0.25, 0.5, 0.75, 1.0, 2.0
2.25	0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.33, 0.5, 1.0, 2.0
3.99	0, 0.1, 0.15, 0.2, 0.25, 0.33, 1.0, 2.0

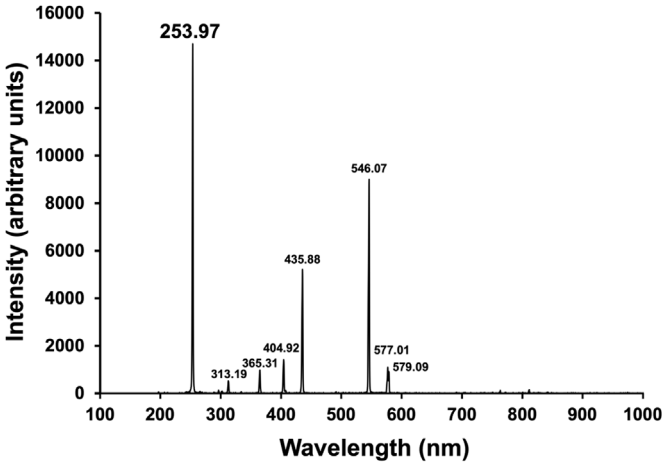

Fig. 1. Emission spectra of a 15-watt lamp sample used as UV-C source in the inactivation studies. Dominant peak at 253.7 nm confirmed UV-C emission.

2.2. Culture maintenance and cocktail inoculum preparation

All 12 challenge organisms are currently maintained in the Laboratory of Food Microbiology and Hygiene (LFMH) Department of Food Science and Nutrition, College of Home Economics, University of the Philippines, Diliman. The strains of *Salmonella enterica* serovars include Typhimurium (American Type Culture Collection, ATCC 14028), Abortus-Equi (ATCC 9842), Enteritidis (LFMH S1-10, S4-18, S5-18, and S6-18), Infantis (LFMH S2-10), Montevideo (LFMH S3-10) and Diarizonae (ATCC 12325 and 29,934), Oranienburg (S8-18), and Senftenberg (S7-18).

Culture maintenance and inoculum preparation protocols previously reported by Gabriel and Colambo (2016) were followed in this study. Working culture slants were prepared by obtaining a loop of cells from each of the stock cultures, and transferring them into 10 mL nutrient broth (NB, HiMedia). The broth cultures were then incubated at 35 °C for 18–24 h. A loop of cells were then obtained from each of the NB cultures and transferred to another 10 mL NB tube for a 2nd culture passage. Finally, working cultures were prepared by obtaining cells from the 2nd culture passage and streaking onto nutrient agar (NA, HiMedia) slants prior to incubating at 35 °C for 18–24 h. The resulting working cultures were then stored at 4 °C until used in the challenge

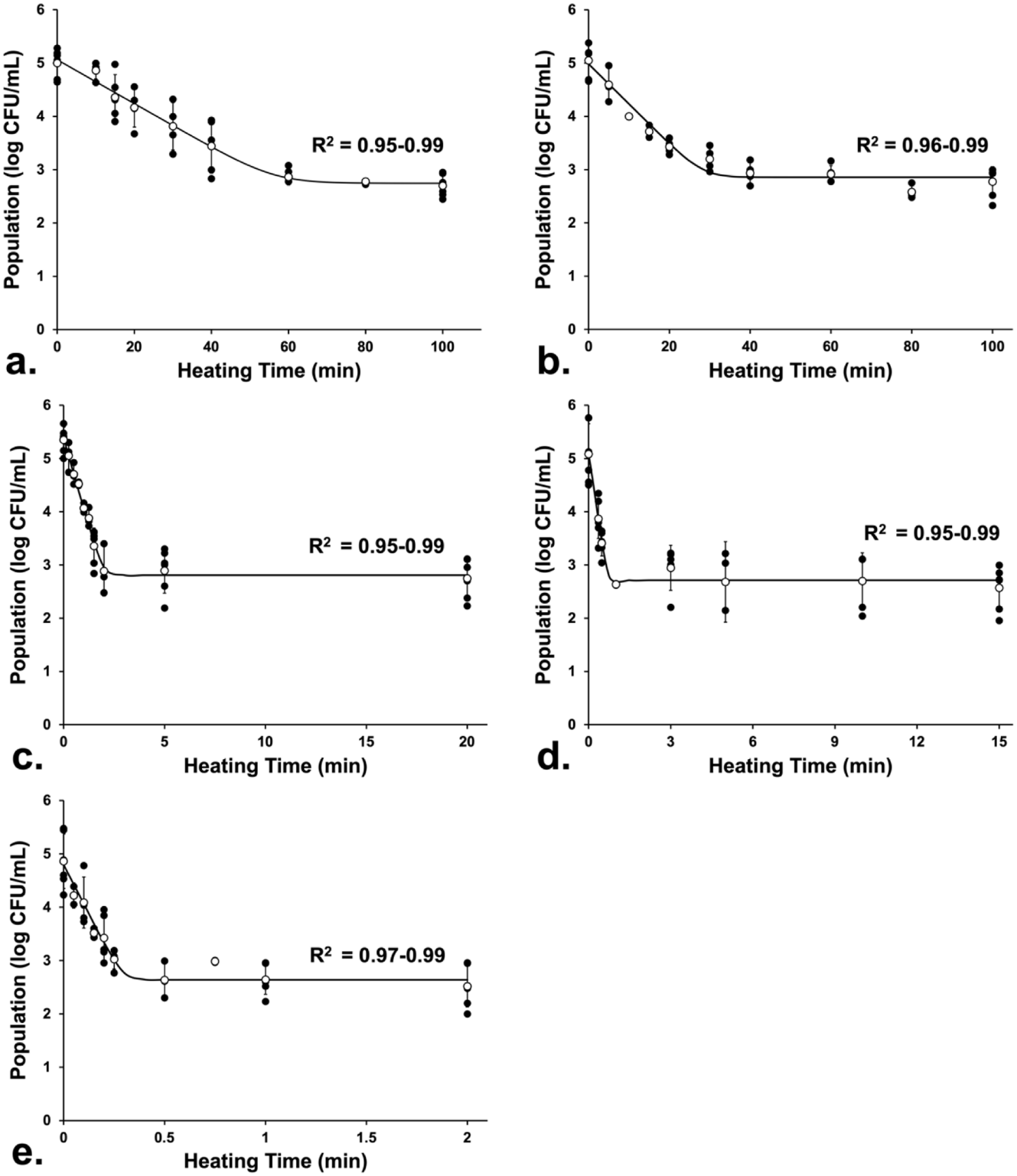

Fig. 2. Thermal inactivation curves of a cocktail of *Salmonella enterica* in cold-pressed VCO subjected to various heating temperatures (a.) 50, (b.) 55, (c.) 60 (d.) 65 and (e.) 70 °C. Filled markers represent populations enumerated in independently replicated runs. Hollow markers denote average populations that were fitted to the Baranyi and Roberts (1994) to generate representative inactivation curves. The R^2 values presented are ranges obtained from all the trials conducted, and is a measure of the goodness-of-fit of the curves to the Baranyi and Roberts (1994) model.

studies. This culture maintenance protocol was repeated every 7 d to ensure that all challenge organisms were of the same culture age and physiology.

In the preparation of inoculum, cells from each of the working cultures were obtained and subjected to the previously described 2-culture passage protocol. A cocktail of the 12 *S. enterica* isolates were prepared by obtaining 1 mL aliquot from each of the 2nd NB culture passage with approximately 7.0–8.0 log CFU/mL, and mixing them in a sterile Erlenmeyer flask. The mixture was then vortexed mixed for 3° s to ensure homogeneity. To harvest cells from the mixture, 1 mL aliquots

Table 2
Inactivation kinetic parameters[a] of *Salmonella enterica* in cold-pressed VCO subjected to heating.

Heating Temperature (°C)	Inactivation Parameters[a]							
	D-values[b] (min)	LR1[c] (log CFU/mL)	t_{LR1}[d] (min)	LR2[e] (log CFU/mL)	TLR[f] (log CFU/mL)	t_{TLR}[g] (min)	Viable Pop[h] (log CFU/mL)	R[b]
50	23.53 ± 4.07[a]	2.17 ± 0.24[a]	58.33 ± 14.38[a]	0.13 ± 0.07[a]	2.30 ± 0.19[a]	100.0	2.60 ± 0.39[a]	0.95–0.99
55	14.45 ± 4.20[b]	2.07 ± 0.42[a]	30.00 ± 8.94[b]	0.21 ± 0.12[a]	2.28 ± 0.50[a]	100.0	2.73 ± 0.32[a]	0.96–0.99
60	0.73 ± 0.15[c]	2.39 ± 0.26[a]	2.00 ± 0.00[c]	0.21 ± 0.16[a]	2.60 ± 0.25[a]	20.0	3.20 ± 1.40[a]	0.95–0.99
65	0.32 ± 0.12[c]	2.28 ± 0.38[a]	1.25 ± 0.39[c]	0.23 ± 0.09[a]	2.51 ± 0.40[a]	15.0	2.57 ± 0.31[a]	0.95–0.99
70	0.14 ± 0.04[c]	2.19 ± 0.78[a]	0.33 ± 0.00[c]	0.15 ± 0.13[a]	2.35 ± 0.88[a]	2.0	2.52 ± 0.36[a]	0.97–0.99

[a, b, c] Values on the same column followed by the same letter are not significantly different at 95% level of significance.
[a] Inactivation parameters are reported as averages of 6 readings ± SD obtained from 3 independently replicated experiments.
[b] D-values refer to decimal reduction times calculated from the initial, logarithmic linear population decline. The D-value is mathematically equivalent to the negative inverse of the slope of the log-linear phase of the inactivation curves, and is equal to the length of time of heating at a specific temperature that will result in 90% (1 log) reduction in the viability of the challenge organism.
[c] Population decline achieved after the log-linear inactivation phase.
[d] The length of time it took to achieve LR1.
[e] Additional population change in during the inactivation tail.
[f] The total population change achieved throughout the heating process, *i.e.,* LR1 + LR2.
[g] The length of time it took to achieve TLR.
[h] Viable Populations were determined by obtaining the difference between the initial population and the TLR.

were obtained from the cocktail and spun at 2400 × *g* for 10 min using a high-speed bench top centrifuge (Cole Parmer, USA). To ensure homogenous mixing of the inoculum with the suspending medium during ther thermal inactivation studies, the supernatant liquids were thereafter decanted, and allowed to dry in a laminar flow hood for 30 min. The pelletized cells were resuspended in 1. o mL VCO by vortex mixing for 1 min. The cells were acclimatized in VCO for 15 min prior to the inactivation studies.

2.3. Thermal inactivation

For the thermal inactivation studies, 9.9 mL VCO were dispensed into sterile test tubes and immersed in a water bath (Lab Companion, Batch Circulator, Jejo Tech, Korea) set to heat the oil to 50, 55, 60, 65 or 70 °C. For temperature monitoring, a thermometer was inserted through the cold point of a separate tube. This was located slightly below the geometric center of the heated VCO, although the temperature differences recorded were < 0.2 °C (data not presented). Once the desired temperature was reached, 0.1 mL of the cell suspension in VCO was introduced to the heated oil, with constant agitation to make sure that the cells were homogenously introduced to the heated medium. Splattering on the tube walls were minimized in the process. The introduction of the inoculum and the agitation during heating did not result in significant change in the heated oil temperature (data not presented). The cells were subjected to heat for predetermined time periods (Table 1). The inoculated tubes were constantly manually agitated throughout the heating process, without any observable changes in the heating temperature. After heating, the tubes were immediately withdrawn from the water bath and subjected to 10-fold serial dilution with 0.1% peptone water (PW, HiMedia) supplemented with 1% Tween 80 (Labchem, Ajax Finechem, Australia). The supplementation of Tween 80 to the diluent allowed for better enumeration of cells when the VCO were diluted with the aqueous PW. This was similarly done by Murphy (2016) for thermal inactivation of *S. enterica* and *E. coli* in recycled cooking oil. Appropriate dilutions were surface-plated onto pre-solidified NA plates, which were then incubated at 35 °C prior to colony enumeration.

2.4. Ultraviolet-c irradiation

For the ultraviolet-c irradiation challenge studies, cells were first propagated and harvested, and cocktailed in VCO following the previously described protocols. Acclimatized cells in 0.04 mL oil were introduced into aliquots of 4.96 mL uninoculated VCO in 60 mm Petri Dishes, which were thereafter placed on a magnetic stirrer and in a fabricated UV-C box containing multiple 15-W UV-C lamps (G15T8, Sankyo Denki, Tokyo, Japan), which can individually be turned on to control the UV-C irradiance received at the surface of the treated VCO. The total liquid thickness in the dish was 0.7 cm, while the lamp to liquid surface distance was 10 cm. Prior to experimentations, the predominant emission of the UV-C lamps were confirmed using UVX radiometer (Ultra-Violet Products Ltd., Cambridge CB4 1 TG UK) as presented in Fig. 1. In the UV-C irradiation challenges, cells were exposed to 1.25, 2.25 and 3.99 mW/cm^2 for predetermined time periods (Table 1) while vigorously stirring using a pre-sterilized 1.25 cm spin bar to maximize the biocidal activity of UV-C. The VCO was stirred throughout the irradiation process. The UV-C irradiations were conducted at 25 °C. Survivor populations were determined following previously discussed protocols using Tween 80-supplemented PW diluent and NA growth medium. Plates were similarly incubated at 35 °C prior to colony enumeration.

2.5. Inactivation behavior and kinetic parameter determinations

Enumerated survivor populations were expressed as log CFU/mL. Using the freeware DMFit (Version 2.1) from the Institute of Food Research, Reading, UK, the inactivation behaviors of the test organism in the heated and UV-C irradiated VCO were visualized by fitting the survivor populations into the model developed by Baranyi and Roberts (1994). Furthermore, the inactivation kinetic parameters were determined from the curves generated by the DMFit freeware. The Baranyi and Roberts (1994) model was originally developed to determined microbial growth curves, but can also be used to model sigmodal inactivation curves by fitting in the following equation as similarly demonstrated by Xiong, Xie, Edmondson, Linton, and Sheard (1999). N and N_0 are the microbial populations (log CFU/g) at times t and zero (min), respectively; while $-k_{max}$ is the maximum death rate (log CFU/min). If the organism demonstrated initial resistance towards the inactivating agent, α (t) is the inactivation lag/shoulder adjustment function; and β (t) is the tailing adjustment function. Further derivations of the this equation are presented by Xiong et al. (1999).

$$\frac{dN}{dt} = -k_{max}\alpha(t)N\beta(t) \quad (N > 0;\ t \geq 0)$$

$$N(0) = N_0 \quad (N_0 > 0;\ t = 0)$$

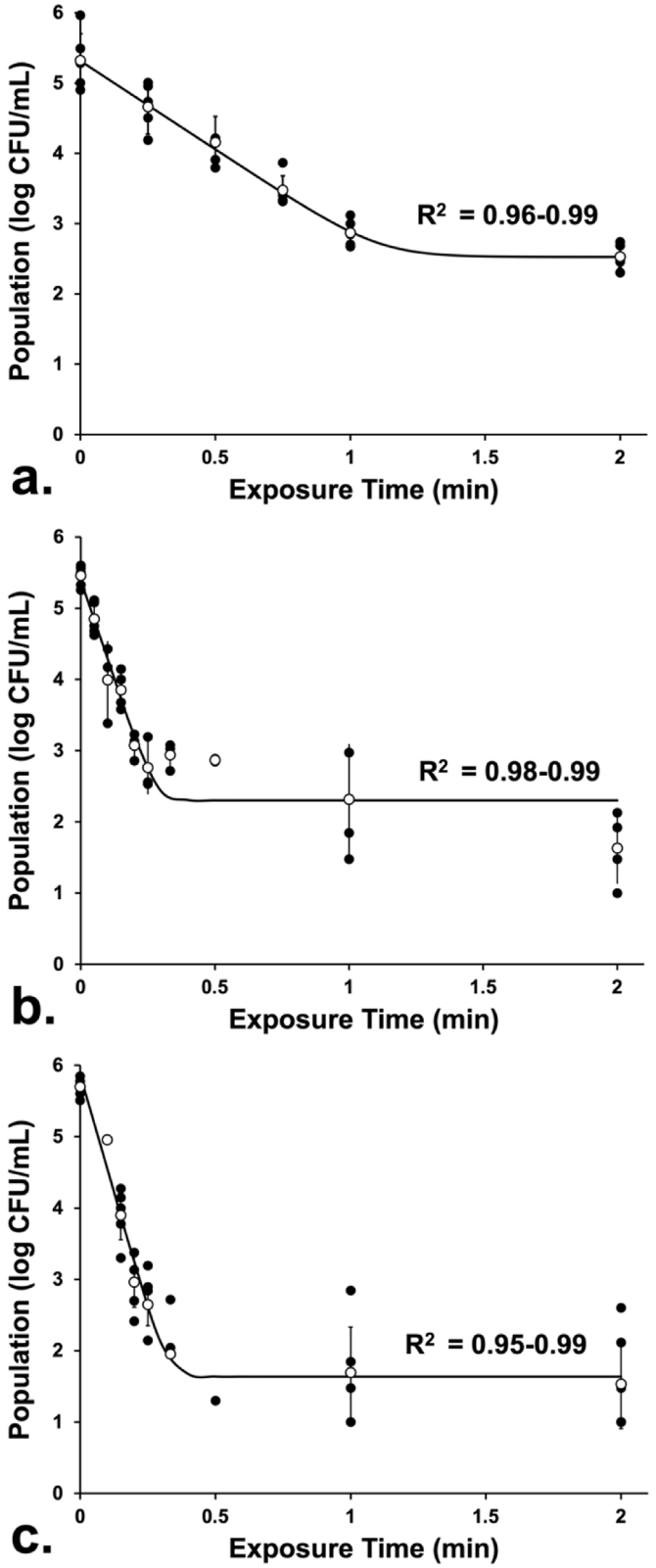

Fig. 3. UV-C inactivation curves of a cocktail of *Salmonella enterica* in cold-pressed VCO subjected to various irradiance (a.) 1.25 ± 0.02 (b.) 2.25 ± 0.02 and (c.) 3.99 ± 0.01 mW/cm^2 Filled markers represent populations enumerated in independently replicated runs. Hollow markers denote average populations that were fitted to the Baranyi and Roberts (1994) to generate representative inactivation curves. The R^2 values presented are ranges obtained from all the trials conducted, and is a measure of the goodness-of-fit of the curves to the Baranyi and Roberts (1994) model.

2.6. Statistical analyses

All inactivation studies were conducted with a minimum of 3 independent runs, with each run having 2 internal replications. Inactivation kinetic parameters were subjected to Analysis of Variance (ANOVA) using the general linear model procedure (PROC GLM) of Statistical Analysis Systems (SAS Institute, version 8.0). The Duncan's Multiple Range Test (DMRT) was used for post-hoc analysis when significant differences existed among values ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical and microbiological properties of cold-pressed VCO

Results of the physicochemical property determinations (data not presented) showed that the test cold-pressed VCO had an average and standard deviation pH value of 7.43 ± 0.40. The free fatty acid content was 0.25 ± 0.06, which was slightly higher than the 0.20% maximum value set by the Philippine National Standards (BPS-DTI, 2007). The UV-C absorbance of the VCO was 1.74 ± 0.01, which has an equivalent UV-C transmittance value of 1.82 ± 0.05%. This UV-C-absorbing property of coconut oil was similarly reported by Widiyati (2017). The total aerobic plate count and yeast and molds count of the VCO samples were also found to be both at < 1.0 log CFU/mL.

3.2. Thermal inactivation of S. enterica

The thermal inactivation curves of the cocktail of *S. enterica* in VCO subjected to varying heating temperatures are presented in Fig. 2a–e. These plots show that the inactivation behavior of the test organism followed a characteristic pattern composed of two phases that fit in curves with upward concavity. In all heating temperatures and exposure time periods, the enumerated populations significantly fitted into the Baranyi and Roberts (1994) model, with R^2 values ranging from 0.95 to 0.99. The initial phase can be described by a rapid, logarithmic linear population decline, which is followed by an inactivation tail phase where very minimal population reductions take place. This inactivation pattern is contrary to the monophasic log-linear inactivation curve described by Moats (1971) for bacteria in heated food systems. Peleg (2006) explained that in this inactivation pattern, the microorganisms have heterogeneous resistance towards the inactivating agent. The log-linear population decline represents the inactivation of sensitive members of the population. As the destruction process proceeds, progressively sturdier survivors are left behind in the treated food system. Hence, the inactivation tail represents the phase where longer time is necessary to destroy the same fraction of the remaining population. Forsythe (2000) also explained that cell clumping can also cause deviation of microbial inactivation behavior from the non-linear pattern. Murphy (2016) similarly reported this biphasic inactivation behavior when *S. enterica* and *E. coli* O157:H7 were suspended in used cooking oil heated to 62, 71, and 82 °C. Xu et al. (2019) determined the thermal inactivation kinetics of *S.* Enteritidis PT30 in low water activity foods including wheat flour, almond flour, and whey protein and reported that in such food systems, changes in water activity during heating influence the thermal resistance of the organism. In this study, water activity changes in the heated VCO were not monitored, and their effects of thermal resistance were not determined. These can be further explored in furture works to better understand the behavior or the target organisms and the efficacy of the killing agent.

The thermal inactivation kinetic parameters determined from the inactivation curves are summarized in Table 2. The decimal reduction times (*D*-values) determined in the log-linear inactivation phase of microbial death was inversely proportionate with the heating temperature. An increase in the heating temperature from 50 to 55 °C resulted in significant ($p < 0.05$) reduction in resistance, with almost 40% increase in the *D*-value of *S. enterica.* Further increase of heating

Table 3
Inactivation kinetic parameters[a] of *Salmonella enterica* in cold-pressed VCO subjected to UV-C irradiation.

Irradiance (mW/cm[b])	Inactivation Parameters[a]								
	D-values[b] (min)	D_{UV-C}[c] (mJ/cm[b])	LR1[d] (log CFU/mL)	t_{LR1}[e] (min)	LR2[f] (log CFU/mL)	TLR[g] (log CFU/mL)	t_{TLR}[h] (min)	Viable Pop[i] (log CFU/mL)	R[b]
1.25 ± 0.02	0.41 ± 0.08[a]	30.57 ± 6.33[a]	2.59 ± 0.40[b]	1.20 ± 0.19[a]	0.09 ± 0.10[a]	2.68 ± 0.36[b]	2.0	2.64 ± 0.11[a]	0.96–0.99
2.25 ± 0.02	0.08 ± 0.02[b]	10.92 ± 0.07[c]	3.22 ± 0.77[ab]	0.33 ± 0.16[b]	0.16 ± 0.16[a]	3.38 ± 0.92[a]	2.0	2.08 ± 0.88[ab]	0.98–0.99
3.99 ± 0.01	0.08 ± 0.02[b]	19.32 ± 0.28[b]	4.00 ± 0.71[a]	0.47 ± 0.21[b]	0.16 ± 0.10[a]	4.17 ± 0.69[a]	2.0	1.53 ± 0.71[b]	0.95–0.99

[a, b, c] Values on the same column followed by the same letter are not significantly different at 95% level of significance.
[a] Inactivation parameters are reported as averages of 6 readings ± SD obtained from 3 independently replicated experiments.
[b] D-values refer to decimal reduction times calculated from the initial, logarithmic linear population decline. The D-value is mathematically equivalent to the negative inverse of the slope of the log-linear phase of the inactivation curves, and is equal to the length of time of heating at a specific irradiance that will result in 90% (1 log) reduction in the viability of the challenge organism.
[c] D_{UV-C} is the amount of UV-C energy or the UV-C dose needed to inactivate the challenge organism by 90% (1 log).
[d] Population decline achieved after the log-linear inactivation phase.
[e] The length of time it took to achieve LR1.
[f] Additional population change in during the inactivation tail.
[g] The total population change achieved throughout the heating process, *i.e.,* LR1 + LR2.
[h] The length of time it took to achieve TLR.
[i] Viable Populations were determined by obtaining the difference between the initial population and the TLR.

temperature to 60 °C resulted in > 30-fold reduction in the *D*-value of the test organism. However no further significant change in the thermal resistance was observed from 60 to 70 °C. Murphy (2016) similarly determined the *D*-values of 5 strains of *S. enterica* in cooking oil between 62 and 81 °C. This previous work reported *D*-values determined on non-selective medium ranging from 0.04 to 1.34 min, and on selective medium ranging from 0.04 to 0.79 min. These values comparable to those determined in this current study, which are summarized in Table 2. It is interesting to note that despite the significant variation in the calculated *D*-values, the population change during the log-linear decline (LR1) across the heating temperatures were not significant ($p > 0.05$) (2.07–2.39 log CFU/mL). The observed population change was however achieved for a heating time ranging from 0.33 to 58.33 min. These results showed that the lethal rates against *S. enterica* achieved by heating at 50 °C for 58.33 min, and at 70 °C for 0.33 min are equal. All the other inactivation parameters, namely the additional population change during the inactivation tail (LR2), the total population reduction (TLR = LR1 + LR2), and resistant, non-inactivated populations were no longer significantly different across heating time.

3.3. UV-C inactivation of S. enterica

Fig. 3a–c presents the inactivation curves of *S. enterica* in VCO subjected to increasing UV-C irradiance. These curves also show that the test organism exhibited the biphasic inactivation behavior consisted of initial logarithmic-linear population decline, followed by inactivation tail. The enumerated survivor populations throughout the exposure period in all irradiance values significantly fitted into the Baranyi and Roberts (1994) model, evidenced in the R^2 values, which ranged from 0.95 to 0.99. Sastry, Datta, and Worobo (2000) explained that this inactivation pattern is common among microorganisms subjected to UV-C irradiation. In fact, *S. enterica, L. monocytogenes* and the innate microflora of a number of solid food products exhibited this inactivation behavior upon subjection to UV-C irradiation (Cheigh, Hwang, 2013 and Dogu-Baykut et al., 2014; Gabriel et al., 2017). Gabriel and Marquez (2017) reported the same inactivation behavior for a number of organisms including *E. coli* O157:H7, *P. aeruginosa, L. monocytogenes,* and *S. aureus* in UV-C irradiated human donor milk.

As expected, increasing UV-C irradiance delivered onto the surface of the vigorously stirred VCO resulted in decreasing *D*-values (min) of the test organism. However, the *D*-values determined when the irradiance were increased to 2.25 and 3.99 mW/cm^2 were no longer significant ($p > 0.05$). The highest initial population decline (LR1) was 4.0 log CFU/mL in the highest irradiance setting, which was also not different from the population change achieved in the mid setting. The total population of *S. enterica* inactivated (TLR) ranged from 2.68 to 4.17 log CFU/mL, which were all achieved within 2 min of irradiation. The inactivation kinetic parameters determined for *S. enterica* in VCO in this study showed that the organism exhibited less resistance in the oil compared to that in UV-C irradiated human donor milk (surface irradiance 1.37 mW/cm^2) (Gabriel & Marquez, 2017). The discrepancy in the resistance may be attributed to the difference in the properties of the suspending medium. Despite the high UV-C absorbance of VCO, the absence of suspended particulates such as proteins and dissolved sugars that further reduce the efficacy of UV-C must have enhanced the anti-*Salmonella* effect in VCO. The study did not determine the exact composition of the VCO but according to the declared nutrition facts, the product was devoid of sugar, fiber, and protein. Furthermore, according to the Philippine National Standards (BPS-DTI, 2007; DA-PCA, 2005), VCO is only allowed to have a maximum of 0.20% moisture and volatile content, and 0.20% free fatty acids. Food additive supplementation is also not allowed.

Comparing the efficacies of thermal treatment and UV-C irradiation as summarized in Tables 2 and 3, it is apparent that the tested UV-C settings were exhibited greater anti-*Salmonella* activity than any of the heating temperatures. Greater population changes were achieved in the UV-C-irradiated VCO at shorter treatment times. Nevertheless, complete inactivation of the pathogens was not achieved in any of the treatments tested, emphasizing the importance of having food safety systems to be in place in the production line to avoid high contamination levels (5.0–6.0 log CFU/mL). Furthermore, despite the observed efficacy of UV-C treatment in eliminating *S. enterica* in VCO, limitations in the application of UV-C as a decontamination process in the industrial scale should be considered and optimized. This should consider the poor penetrative power of UV-C, which may be enhanced by introduction of turbulence through proper machine design. Such considerations may also be applied for the extraction and processing of other high-value products such as essential oils and flavor components from herbs and spices.

4. Conclusion and recommendations

Results obtained in this study demonstrated the resistance of *S. enterica* in VCO towards heat and UV-C, evidenced in the inactivation pattern and kinetic parameters. Comparing the inactivating agents, shorter treatment times resulted in greater population decline in UV-C irradiated VCO. Higher heating temperatures were not tested in this study as this may affect the sensory profile unique to cold-pressed

VCOs. In both heated and irradiated samples, the observed biphasic inactivation behavior, and the inability of any of the tested treatment to completely eliminate the pathogen may be due to the high contamination level tested. Future works should investigate the effect of contamination level on inactivation behavior and kinetic parameters. The effects of both thermal and UV-C treatments on the physicochemical and sensory quality attributes of VCO is also being recommended to further assess the utility of these decontamination processes in improving the safety of the commodity. The results determined in this study also underscore the importance of implementing food safety systems such as Good Manufacturing Practices and Hazard Analysis Critical Control Point in the production of VCO that lack kill steps against pathogenic microorganisms that may highly contaminate the raw materials or finished products during the process flow.

CRediT authorship contribution statement

Alonzo A. Gabriel: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Israel N. Nepomuceno:** Formal analysis, Writing - original draft.

Declaration of competing interest

The authors whose names are listed above certify that they have no affiliations and relationships with any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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