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Microbial shelf life of coconut water subjected to various inoculation levels of *Listeria monocytogenes* and storage conditions

Richard Paolo M. Aba [*], Eric Miguel L. Gelido, Kattleya Marie Reine S. Yatco, Alonzo A. Gabriel

Laboratory of Food Microbiology and Hygiene, College of Home Economics, Alonso Hall, A. Ma. Regidor St., University of the Philippines, Diliman, Quezon City 1101, Philippines

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ABSTRACT

The study determined the growth kinetic parameters of a cocktail of *Listeria monocytogenes* 1/2c and 4b strains in coconut water (pH 4.76, 5.0°Brix, 0.09% malic acid, a_w 0.998) subjected to low (~2.0 log CFU/mL) and high (~4.0 log CFU/mL) contamination levels, and exposed to different storage temperatures (4 °C, 17 °C, 30 °C, and 35 °C). The pathogen proliferated in all tested conditions except in that with low contamination stored at 4 °C. Despite not growing at 4 °C, the pathogen was detectable throughout the storage period, which lasted for almost 400 h. In conditions where the pathogens proliferated, growth lag (t_{lag}) ranged from 0.0 to 68.3 h. The growth rates (K_G) ranged from 0.05 to 0.48 log CFU/h, while the final populations ranged from 6.3 to 8.7 log CFU/mL. Both storage temperature and contamination level significantly ($P < 0.05$) affected the growth parameters. Sanitary risk times (SRT) were determined with the microbiological shelf life (SL) of coconut water. In some of the conditions tested, SRT took place before SL (SRT < SL), emphasizing the importance of having good hygienic and manufacturing practices in place for such a vulnerable commodity.

1. Introduction

The consumer preference shift towards healthier, minimally processed products such as fruit juices may be attributed to the campaigns of the government, and the industry; and increasing consumer health consciousness (AIJN European Fruit Juice Association, 2016). Aside from health benefits, convenience, accessibility, and affordability were also previously reported to be reasons for consumer patronage of fruit juices (Gabriel et al., 2005). However, this preference shift has also increased consumer vulnerability towards infections of pathogenic microorganisms (Callejon et al., 2015; Gabriel, 2014; Noel et al., 2010). In the Netherlands, an outbreak of *Salmonella enterica* serovar Panama gastroenteritis involving mostly young adult women was linked with the consumption of unpasteurized fresh fruit juices (Noel et al., 2010).

In the Philippines, one of the most commonly consumed fruit juices is fresh, unprocessed green coconut water. The liquid endosperm obtained from young (6 to 9 mos) green coconuts is locally called *buko* juice, which refers to the unmodified natural aqueous liquid that is clear to slightly turbid in appearance (PNS-BAFPS, 2006). Coconut water is high in potassium but low in sodium (PNS-BAFPS, 2008), with approximately 5% sugar, 0.7% protein, and 0.2% fat (FAO, 2007). With the nutritional value of coconut water, the FAO (2007) regarded it as a refreshing sports drink with wide marketability. In terms of safety, its intrinsic properties and the manual extraction process of coconut water make the commodity prone to microbial contamination (Gabriel et al., 2016). While it has been reported that coconut water is sterile (Maciel et al., 1992; Piló et al., 2009; PNS-BAFS, 2016), contamination may come from the surface of the coconut drupes, pieces of equipment and utensils, food handlers, and immediate environment due to poor hygienic practices during extraction, packaging, and distribution (Awua et al., 2011; Kajs et al., 1976; Piló et al., 2009; Sinigaglia et al., 2006; Walter et al., 2009; Ziegler et al., 2018).

There have been no outbreak reports from the consumption of coconut water. However, some coconut products were reported to cause foodborne diseases. In 1993, a cholera outbreak emerged in the United States from imported coconut milk from Thailand. More recently, in 2017, the US Food and Drug Association (US FDA) reported multistate outbreaks were caused by *Salmonella* isolated from frozen shredded coconut (US FDA, 2018) and coconut meat pieces (Luna et al., 2018). Despite not being commonly linked with outbreaks related to fruit juices, the US FDA and the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) have also proposed *Listeria*

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[*] Corresponding author at: Laboratory of Food Microbiology and Hygiene, Department of Food Science and Nutrition, Teodora Alonso Hall, College of Home Economics, A. Ma. Regidor Street, University of the Philippines, Diliman Campus, 1101 Quezon City, Philippines.
E-mail address: rpmaba@gmail.com (R.P.M. Aba).

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monocytogenes to be a pertinent pathogen due to its ubiquity and the threat that it poses to vulnerable consumer groups (Register, 2001; US FDA, 2004). Pregnant women, newborns, older adults, and people with weakened immune systems are at risk of listeriosis, which may lead to sepsis or meningitis (CDC, 2016a; Farber and Peterkin, 1991). Refrigeration is a common method of extending the shelf life of minimally processed commodities. However, psychrotrophic microorganisms such as *L. monocytogenes* can survive and grow at a wide temperature range (Gandhi and Chikindas, 2007; Sinigaglia et al., 2006; Walter et al., 2009). Aside from this, *L. monocytogenes* have also been reported to tolerate a wide pH range, including those of fruits and vegetables (Farber and Peterkin, 1991; Keller and Miller, 2005). Walter et al. (2009) reported that *L. monocytogenes* proliferated in fresh coconut water stored at 4 °C, 10 °C, and 35 °C. The refrigeration at 4 °C and 10 °C retarded the growth of *L. monocytogenes*. At 35 °C, the lag time for this microorganism was reduced.

The FAO-WHO (2004) conducted a microbiological risk assessment for *L. monocytogenes* in selected foods. Results showed that the risk associated for potential growth of the pathogen is affected by the food matrix and environmental conditions such as storage temperature and time. Understanding the crucial interactions of food-, environment-, and microorganism-specific variables may lead to the reduction of risk associated with *L. monocytogenes.* Thus, considering the current consumer demands for and market potential of coconut water and its vulnerability to microbial contamination including the ubiquitous *L. monocytogenes*, this study was conducted to determine survivability and growth kinetic parameters of *L. monocytogenes* in young coconut water subjected to different storage conditions. Results obtained in the study can be useful for better control of the safety of the commodity.

2. Materials and methods

2.1. Green coconut water

Green coconut water samples were obtained from a local distributor in Krus na Ligas, Quezon City, Philippines. The samples were obtained by successive cutting on the drupes until the mesocarp was exposed. A small hole was made through the endocarp to allow aspiration of the liquid endosperm (coconut water). The samples were transferred into a sterile media bottle placed in an icebox and immediately transported to the laboratory. The samples were filter-sterilized through a 0.45 μm mixed cellulose ester membrane filter (Advantec, Toyo Roshi Kaisha Ltd., Japan). Sterility was confirmed by surface plating onto Plate Count Agar (HiMedia, Mumbai, India) at 35 °C for 24 h, and acidified Potato Dextrose Agar (PDA, HiMedia) 28 °C for 72 h to determine the total aerobic mesophilic bacterial count (TAMBC) and yeast and molds count (YMC), respectively.

Filter-sterilized coconut water with a volume of 148.5 mL was distributed in identical sterile media bottles and kept under refrigerated conditions for not more than 12 h prior to being used in the inoculation and population monitoring studies. Furthermore, the total soluble solids (TSS, °Bx) and the pH of the sample were obtained using a handheld refractometer (Atago, Tokyo, Japan) and benchtop pH meter (Horiba LAqua F71, Tokyo, Japan), respectively. Titratable acidity (TA, % malic acid) was determined using potentiometric titration with standardized 0.1 N sodium hydroxide (NaOH, RCI Labscan, Thailand) until the pH 8.2 endpoint. Finally, water activity (a_w) was determined using a Novasina ms1 set aw (Novasina, Pfaffikon, Switzerland) following manufacturer-detailed procedures. All physicochemical property analyses were conducted in triplicate.

2.2. Listeria monocytogenes and cocktail inoculum preparation

Two strains of *Listeria monocytogenes* maintained at the Laboratory of Food Microbiology and Hygiene (LFMH), University of the Philippines Diliman (1/2c LFMH L1–10 and 4b L2–10) were used in this study. Both strains were isolated from clinically-derived samples in Hiroshima City Institute of Public Health, Japan. A loopful of cells were obtained from refrigerated working nutrient agar (NA, HiMedia) slants, activated by transferring a loop of inoculum into a 10 mL nutrient broth (NB, HiMedia), and were incubated at 35 °C for 24 h. Each activated strain was subjected to a 2nd culture passage by obtaining a loopful of inoculum from the 1st passage, transferring into a new NB tube, and incubating for another 24 h. A cocktail of the 2 test strains was prepared by mixing equal volumes of the 2nd passage NB culture in a sterile flask, and vortex mixing for 30 s. Cells were harvested from the culture broth by obtaining 1 mL aliquot and transferring in a microcentrifuge tube. The tube was spun in a benchtop microcentrifuge (Cole Parmer, USA) at 2400 *x g* for 15 min. The supernatant was discarded and was replaced with one (1) mL sterile coconut water. The tube was vortexed for 30 s (s) to ensure proper cell suspension and minimize cell clumping. The suspended inoculum was acclimatized at ambient condition for 10 min prior to inoculation to green coconut water.

2.3. Contamination levels and storage temperatures

The pooled *L. monocytogenes* strains were subjected to 10-fold serial dilutions with sterile coconut water until the desired population densities were reached. Pooling was performed to simulate the presence of multiple strains of microorganisms present in food samples. A final dilution was made by introducing of 1.5 mL of the cell suspension into the previously dispensed 148.5 mL in media bottles. In this study, low (~2.0 log CFU/mL) and high (~4.0 log CFU/mL) inoculation levels were introduced to the filter-sterilized coconut water prior to incubation at different storage temperatures. The initial population was determined by surface plating the sample on NA. When the desired initial population was achieved, the experiment proceeded with plating at different time intervals.

The tested storage temperature included 4 °C, based on the recommended storage condition for ready-to-eat food products (FSANZ, 2019), while 17 °C represents a malfunctioning refrigeration unit. The 30 °C storage temperature is close to the mean annual temperature, and the temperature during the warmest months in the Philippines, which are 26.6 °C, and 28.3 °C, respectively (DOST-PAGASA, n.d.). Finally, the 35 °C test temperature represents extreme weather conditions experienced during *El Niño* (Chiu, 2019). Samples stored at 4.0 °C and 17.0 °C were placed in a water bath circulator (Lab Companion, Jejo Tech, Korea), while those stored at 30 °C and 35 °C were placed in an incubator (Memmert, Germany).

2.4. Population monitoring and model fitting

Enumeration of *L. monocytogenes* colonies were done by plating the sample at predetermined time intervals. One mL aliquots were withdrawn from the samples and were serially diluted using 0.1% peptone water (PW, HiMedia). Diluents were surface plated on pre-solidified NA and were incubated at 35 °C for 24 h. Colonies were expressed as log CFU/mL. The growth curve and growth kinetic parameters were established by fitting the data in a Dynamic Model Fit (DMFit) 3.0 (Institute of Food Research, UK) which was based on the works of Baranyi and Roberts (1994). Growth kinetic parameters generated include lag time (t_{lag}, h), growth rate (K_G, log CFU/h), final population (Pop_{fin}, log CFU/mL), and regression coefficient (R^2). The delay due to the need to adapt of the microorganism or "generation-backlog" (h_0) was calculated by multiplying t_{lag} and K_G (Baranyi and Roberts, 1994).

2.5. Sanitary risk time determination

The sanitary risk time (SRT) for each condition tested were determined using the definition provided by Castillejo Rodriguez et al. (2000) for *L. monocytogenes.* The SRT is the time needed for the microorganism to increase by 2 log (Eq. (1)). The SRT values were determined without

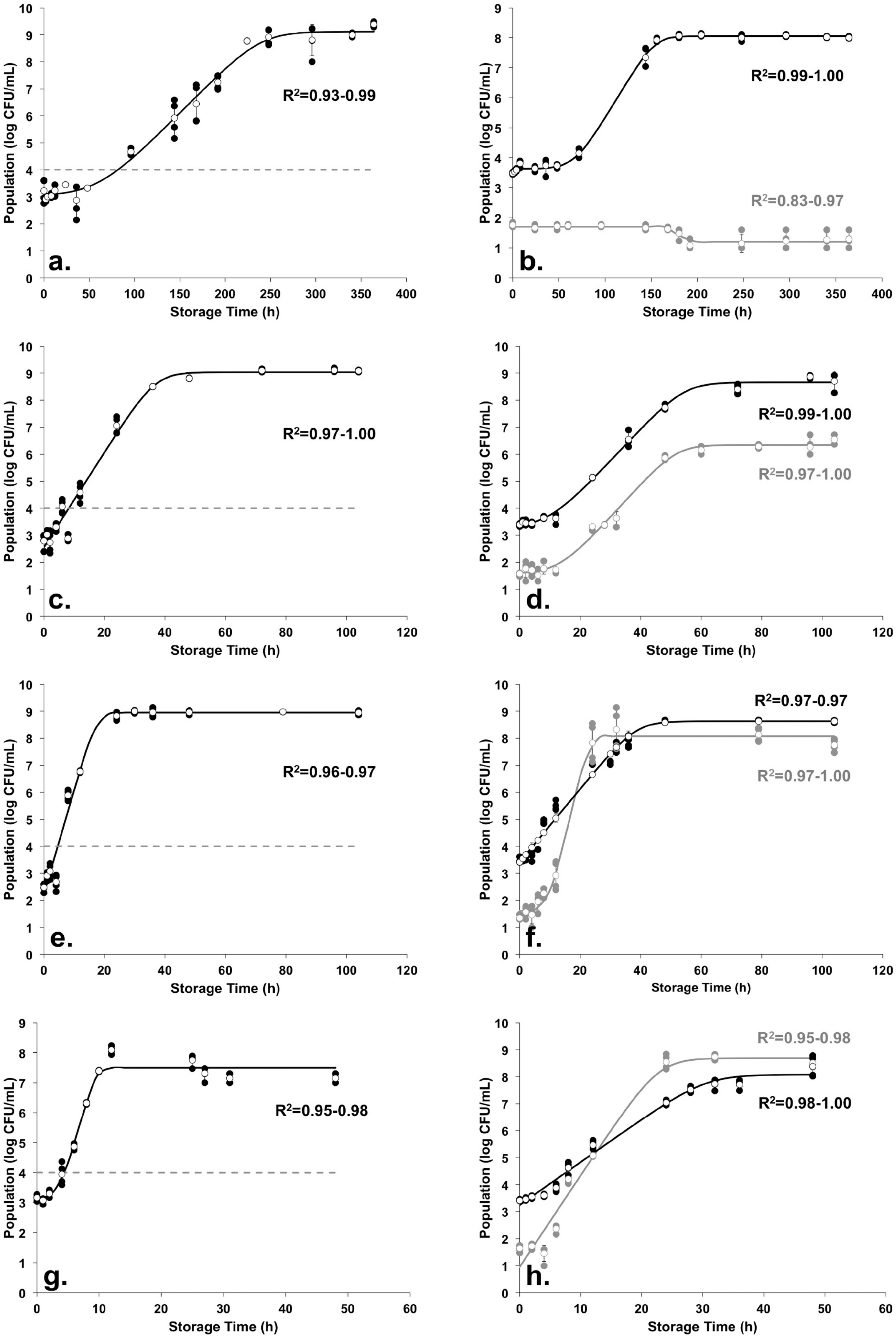

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Fig. 1. Behaviors of autochthonous spoilage microbiota (a, c, e, g) and *Listeria monocytogenes* (b, d, f, h) in coconut water stored at different temperatures (a–b: 4 °C, c–d: 17 °C, e–f: 30 °C, g–h: 35 °C). Contamination levels in b, d, f, h are denoted by grey markers and curves (~2.0 log CFU/mL) and black markers and curves (~4.0 log CFU/mL). Filled markers denote populations obtained from independently replicated experiments, empty markers denote average values. R^2 values measure the goodness-of-fit to the Baranyi and Roberts (1994) growth model. Dashed lines indicate the limit for microbiological standard for the Total Aerobic Mesophilic Bacteria stipulated in the Philippine National Standard for chilled young coconut water/drink (PNS/BAFPS 28:, 2006).

considering the effect of t_{lag}. That is, it was assumed that in worst-case scenarios, the test organism readily multiplies in the food system regardless of the storage temperature and initial population. Moreover, as similarly conducted by Sinigaglia et al. (2006), the relationship between the SRT and storage temperature per contamination level was represented by the linear Eq. (2), where *a* is the theoretical SRT at 0 °C, T is the temperature (°C), and *b* is the slope of the line that represents in the change in SRT for every degree increase in storage temperature. When *b* is multiplied by a factor of 10, the Q_{10} value or the temperature coefficient is determined (Eq. (3)).

$$\mathrm{SRT} = \frac{2\ (log\ \mathrm{CFU})}{K_G(log\mathrm{CFU/h})} \tag{1}$$

$$\mathrm{RT} = a + b\mathrm{T} \tag{2}$$

$$\mathrm{Q}_{10} = 10b \tag{3}$$

2.6. Proliferation of autochthonous microbiota and shelf life of coconut water

Non-filter sterilized and non-*Listeria* inoculated coconut water samples were also subjected to the different test storage conditions to determine the growth kinetic parameters of the autochthonous spoilage microbiota. Aspirated coconut water samples were transferred in sterile media bottles and were stored at different test temperatures. Samples were plated at predetermined time intervals following the previously described plating procedures. The growth kinetic parameters were similarly determined by fitting the populations to the Baranyi and Roberts (1994) model using DMFit. The shelf life (SL) of coconut water was determined in accordance with the specifications of the microbiological standards of the Philippine National Standards of the Bureau of Agriculture and Fisheries Standards, Department of Agriculture, Philippines (PNS-BAFPS, 2006) which was established at 4.0 log CFU/ml. That is, the time needed for autochthonous microbiota to increase by 4 log. Eq. (4) was used to calculate SL.

$$\mathrm{SL} = \frac{4\ (log\ \mathrm{CFU})}{K_G(log\mathrm{CFU/h})} \tag{4}$$

2.7. Statistical analysis

All experiments were composed of two independent runs with two internal replicates. Data calculated were subjected to analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) of the Statistical Analysis Systems (SAS Statistical Software Package v. 8.0, Cary, NC, USA) followed by a post-hoc analysis using Duncan's Multiple Range Test (DMRT) to determine differences at a 95% level of significance.

3. Results and discussion

3.1. Physicochemical properties of background microbiota coconut water

The pH, TSS, TA, and a_w of coconut water were 4.76 ± 0.05, 5.00 ± 0.00 Brix, 0.09 ± 0.01% malic acid, and 0.998 ± 0.00, respectively. These physicochemical properties are within the ranges and therefore conform with those stipulated in the Philippine National Standards (PNS-BAFPS, 2006) for pure coconut water. These properties were also found to be similar to the studies of Gabriel and Arellano (2014), and Tan et al. (2014) for coconut water with different maturity and sources in the Philippines. The pH of coconut water used in this study is also close to other fruits such as bananas, figs, watermelons, and grapes (IFT, 2003). Since coconut water is considered a low acid food, its pH shall not be able to inhibit the growth of *L. monocytogenes* (Sinigaglia et al., 2006). Recently, Nyhan et al. (2018), were able to demonstrate the growth of *L. monocytogenes* across different pH values (pH 4.7–5.3). The TSS and TA of coconut water are significantly lower than unpasteurized orange juice (9.0 to 14.0°Brix, 0.68 to 1.20%citric acid) (Tyl and Sadler, 2017), which is a commonly reported vector of fruit juice-related infections (Vojdani et al., 2008). The a_w of coconut water is within the range for fresh fruit and vegetables, which is also higher than the minimum a_w required by *L. monocytogenes* for growth, both summarized by the IFT (2003). These intrinsic physicochemical properties of coconut water indicate that if the environmental conditions are favorable, *L. monocytogenes* can readily proliferate in the test food system.

3.2. Fates of L. monocytogenes in coconut water

The behaviors of *L. monocytogenes* introduced at low (~2.0 log CFU/mL) and high (~4.0 log CFU/mL) inoculation levels to coconut water, and thereafter exposed to various storage temperatures are summarized in Fig. 1. The Baranyi and Roberts (1994) model was fitted to the enumerated populations. Results in Table 1 showed that the test organism exhibited growth in all conditions tested except when stored at the lowest temperature of 4 °C at a low inoculation level. However, despite the inability of *L. monocytogenes* to grow in such a condition, it can be seen that it remained viable and detectable even after the last sampling time of 364 h, where the average population enumerated was at 1.3 log CFU/mL. Kinetic parameters summarized in Table 1 showed that in conditions where proliferation was observed, growth lag times (t_{lag}) ranged from 0.0 to 68.3 h. The t_{lag} values were significantly affected ($P < 0.05$) by storage temperatures. The significant effect of inoculation level on t_{lag} was only observed at 30 °C. The specific growth rates (K_G) were generally faster at higher storage temperature. A similar trend was also observed for K_G at lower inoculation levels. At 30 °C, the K_G was 4 times faster at lower inoculation level, while at 35 °C, the K_G was twice as fast when the initial population was low. Contois (1957, 1959) demonstrated that bacterial growth rates are affected by population densities in both batch and continuous cultures. The effects of inoculation level and storage temperature on the final population (Pop_{fin}) varied, which ranged from 6.3 to 8.6 log CFU/mL. According to Baranyi and Roberts (1994), the parameter h_0 should be constant when the pre-inoculation of the cells are the same. In reality, this parameter varies. The h_0 was determined in each inoculation level and storage conditions as presented in Table 1. The h_0 is a "generation-backlog" caused by the adaptation of microorganisms prior to its growth. The h_0 values ranged from 0.0 to 6.6 and was observed to be higher at low inoculum levels. The mechanisms of adaptation of *L. monocytogenes* in refrigerated conditions involves the changes in fatty acid composition to restore membrane fluidity, changes in gene expression and induction of cold shock proteins, accumulation of solutes to act as cryoprotectants, and changes in gene transcription by sigma factor B (σ^B) (Gandhi and Chikindas, 2007).

Walter et al. (2009) similarly studied the effects of storage temperature on the growth of a 5-strain cocktail of *L. monocytogenes* in coconut water stored at 4 °C, 10 °C, and 35 °C at an average inoculation level of

Table 1
ComBase-predicted and observed growth kinetic parameters of *Listeria monocytogenes* in coconut water.

Contamination level and storage conditions	Pop_{init} (log CFU/mL)	ComBase-predicted values			Observed values[1]				
		t_{lag} (h)	K_G (log CFU/h)	Pop_{fin} (log CFU/mL)	t_{lag} (h)	K_G (log CFU/h)	Pop_{fin} (log CFU/mL)	h_0[2]	R^2[2]
4.0 °C									
Low contamination	1.8 ± 0.1^{c}	338.8 ± 0.0^{a}	0.005 ± 0.0^{d}	8.5 ± 0.0^{a}	ND	ND	ND	ND	ND
High contamination	3.7 ± 0.5^{a}	338.8 ± 0.0^{a}	0.005 ± 0.0^{d}	8.5 ± 0.0^{a}	68.3 ± 8.5^{b}	0.05 ± 0.01^{e}	8.1 ± 0.0^{b}	$3.6 \pm 0.8^{a}{,}^{b}$	0.99–1.00
17.0 °C									
Low contamination	$1.6 \pm 0.1c^{d}$	37.6 ± 0.0^{b}	0.045 ± 0.0^{c}	8.5 ± 0.0^{a}	13.9 ± 1.5^{c}	0.12 ± 0.01^{d}	6.3 ± 0.2^{c}	6.6 ± 7.4^{a}	0.97–0.99
High contamination	3.4 ± 0.1^{b}	37.6 ± 0.0^{b}	0.045 ± 0.0^{c}	8.5 ± 0.0^{a}	10.0 ± 1.0^{c}	0.12 ± 0.01^{d}	8.6 ± 0.1^{a}	1.0 ± 0.4^{b}	0.99–1.00
30.0 °C									
Low contamination	1.4 ± 0.1^{d}	12.6 ± 0.0^{c}	0.13 ± 0.0^{b}	8.5 ± 0.0^{a}	9.5 ± 1.9^{c}	0.48 ± 0.05^{a}	8.1 ± 0.3^{b}	$4.5 \pm 0.6^{a,b}$	0.97–0.99
High contamination	$3.5 \pm 0.17^{a,b}$	12.6 ± 0.0^{c}	0.13 ± 0.0^{b}	8.5 ± 0.0^{a}	0.0 ± 0.0^{d}	$0.14 \pm 0.00^{c,d}$	8.6 ± 0.1^{a}	0.0 ± 0.0^{b}	0.97–0.99
35.0 °C									
Low contamination	1.6 ± 0.1^{c}	11.1 ± 0.0^{d}	0.15 ± 0.0^{a}	8.5 ± 0.0^{a}	0.0 ± 0.0^{d}	0.34 ± 0.01^{b}	8.7 ± 0.9^{a}	0.0 ± 0.0^{b}	0.95–0.98
High contamination	3.4 ± 0.5^{b}	11.1 ± 0.0^{d}	0.15 ± 0.0^{a}	8.5 ± 0.0^{a}	0.0 ± 0.0^{d}	0.16 ± 0.01^{c}	8.2 ± 0.3^{b}	0.0 ± 0.0^{b}	0.98–0.99

ND. Not determined as the test organisms did not exhibit growth in the experimental condition within the observation period.
a,b,c… Values on the same column followed by the same letters are not significantly different ($P > 0.05$).

[1] Values are reported as averages of 4 readings obtained from 2 independently ran experiments.

[2] h_0 values were calculated by obtaining the product of lag time and growth rate.

3.0 log CFU/mL. In this previous study, t_{lag} and Pop_{fin} were directly proportional, with storage temperature. The opposite relationship was observed for K_G and temperature. However, differences were observed in the kinetic parameters determined in this past study and those established in this current work. For example in this study, at 4 °C, the t_{lag} was >5 times shorter than that observed by Walter et al. (2009). Furthermore in this study, while *L. monocytogenes* immediately proliferated at 35 °C, a t_{lag} of 3.5 h was reported by Walter et al. (2009). The K_G determined in this study at 4 °C was >10 times faster than that previously reported at the same temperature. However, at 35 °C, Walter et al. (2009) reported a K_G that is almost twice as fast as the value determined in this study. This previous work reported Pop_{fin} that ranged from 7.2 to 8.2 log CFU/mL, which were close to those observed in this current study. These observed variations may be due to interstrain differences in the test organisms. Sinigaglia et al. (2006) similarly challenged a single strain of *L. monocytogenes* in coconut meat at refrigerated conditions and at low and high initial population and reported growth in all tested conditions within the 10-d (240-h) observation period. Penteado and Leitão (2004) similarly determined the fates of a single *L. monocytogenes* strain in low-acid fruits (pH 4.87–5.87) including melon, watermelon, and papaya pulps, and reported the direct relationship between storage temperature and organism generation time in all fruits.

3.3. Comparisons of observed behavior with ComBase predictions

The growth kinetic parameters observed for *L. monocytogenes* coconut water was also compared to those predicted by ComBase (2019) repository of growth models in broth systems for an equal number of initial populations, and temperature, pH and a_w settings (Table 1). Results showed that while ComBase (2019) predicted growth for the low contamination level stored at 4 °C, this was not observed in the actual set up. In fact, within the observation period, a decline in the population was observed rather than growth (Fig. 1). Furthermore, based on model predictions, the initial population did not significantly affect t_{lag} and K_G values; and neither the initial population nor storage temperature affected Pop_{fin}. Comparisons of actual and predicted growth kinetic parameters showed that while the model overestimated the t_{lag} of *L. monocytogenes*, it also underestimated the K_G and Pop_{fin} of the test organism. While an underestimation on the K_G and Pop_{fin} were observed, predictive models are often assumed to be conservative. Not all models can predict actual measurements and limitations of the model should be factored in. The discrepancies in the predicted and actual growth kinetic parameters may be attributed to the variation in the characteristics of the organisms the models were established and those used in this study. Furthermore, since the generated model predictions were based on microbial growth in broth systems, the effect of components in a real food system such as the one used in this study could also explain the observed discrepancies.

3.4. Growth of spoilage microbiota, shelf-life and sanitary risk times

The study also determined the microbiological shelf-life (SL) of coconut water stored at varying test temperatures by allowing the autochthonous spoilage microbiota in non-filter sterilized, non-inoculated samples. The SL is defined as the time it takes for the autochthonous spoilage microbiota to reach 4.0 log CFU/mL which is the Total Plate Count limit indicated in the Philippine National Standards for chilled young coconut water/drink (PNS-BAFPS, 2006). Shelf-life is also defined as the unacceptability of a food product based on the changes in its sensory properties (Young and O'Sullivan, 2011). The SL of coconut water was calculated by dividing the maximal log increase (4.0 log CFU/mL) to the growth rate (K_G) of autocthononous spoilage microbiota. The kinetic parameters are summarized in Table 2. Results showed that the initial inherent microbiota of the green coconut water ranged from 2.5 to 3.2 log CFU/mL, which were similar to those reported by Azanza (2005) with aerobic plate counts ranging from 3.0 to 4.0 log CFU/mL. Gabriel et al. (2016) were able to isolate and identify some spoilage microorganisms from young coconut water through 16S rRNA sequencing, which included *Klebsiella* sp., *Kluyvera* sp., and *Staphylococcus epidermidis*. Results summarized in Table 2 showed that enumerated populations over the observation periods fitted by the Baranyi and Roberts (1994) model, generated high R^2 values (0.93–1.00). The growth kinetic parameters of the spoilage microbiota were similarly affected by storage temperature. Increasing temperature from 4 to 17 °C significantly ($P < 0.05$) decreased the t_{lag} of the spoilage microbiota. Beyond 17 °C, no significant changes in the t_{lag} values were observed. Storage temperature and K_G were inversely proportional. The slowest K_G for the spoilage microbiota determined at 4 °C was >20 smaller than the fastest K_G determined at 35 °C. Furthermore, the Pop_{fin} values were directly proportional to storage temperature. These growth kinetic parameters were used in the calculation of microbial shelf-life as indicated in Fig. 1.

It should be noted that in this study, the effects of the presence of autochthonous microbiota on the proliferation of the test pathogen were not determined. According to Jay (2000), the presence of the normal microbiota in food may exhibit general, non-specific inhibition towards an organism. The presence of autochthonous microbiota may become an additional hurdle to the pathogen as a competitor to the nutrients available as they grow faster than the inoculated pathogens (Sinigaglia et al., 2006; IFT, 2002). Therefore, the growth kinetic parameters of *L. monocytogenes* reported in this study may be considered as more conservative and possess additional margins of safety against the pathogen.

3.5. Risk assessment based on sanitary risk time and shelf life

The International Commission on Microbiological Specifications for Foods (ICMSF, 1994) stated that most food products contain low levels (<100 cells/g) of *L. monocytogenes* and listeriosis is of a lesser concern for normal, healthy individuals. However, highly susceptible individuals (elderly, pregnant women, and those in medical treatment) with underlying diseases and reduced immunity have greater risk of acquiring listeriosis (FAO-WHO, 2004). As such, the ICMSF recommended a sampling plan and criteria for *L. monocytogenes* and food exceeding 100 cell/g should be rejected during sampling. The Food Safety Criteria (FSC) and Process Hygiene Criteria (PHC) of the European Union Commission Regulation (EUCR, 2005) has also set in their FSC the level of *L. monocytogenes* < 100 CFU/g. Any ready-to-eat (RTE) food that contains >100 CFU/g is considered a food safety risk and is not compliant by the European Union law. This recommendation by ICMSF and EUCR was used by Castillejo Rodriguez et al. (2000), Sinigaglia et al. (2006), and this current study as the basis for establishing an increase of 2.0 log CFU for sanitary risk assessment. The ICMSF and EU criterion of 100 CFU/g was assumed and furthermore that the initial population of the product is at 1 CFU/g. However, in this study, one of the limitations was inoculation at low levels. Most food samples are inoculated with 100–10,000 CFU/g or even higher (Chen et al., 2016a), hence, an increase by 2 log was used. This is in the assumption that, at worst-case scenario, a low inoculum level represents initial level at 1 log CFU, and the microorganism readily proliferates in test food system. According to Stedefeldt et al. (2018), sanitary risk relates to the possibility of a health threat incident. Understanding sanitary risk in the food service and manufacturing industries involves prediction of health threats, vulnerability of human health, and likelihood of harm.

Several studies reported recent listeriosis outbreaks at low levels in highly susceptible individuals. Some implicated food products include peaches (Chen et al., 2016b), celery (Gaul et al., 2012), caramel apples CDC, 2015), and uncooked frozen vegetables (CDC, 2016b). Buchanan et al. (2017) stated that several researches have concluded that it is impossible to permanently remove *L. monocytogenes* from food because of its ubiquity; thus, elimination and exclusion of this pathogen must be managed by good manufacturing practices (Farber et al., 2020).

A study on dose-response by Pouillot et al. (2015) reported that most foods were contaminated with medium to high concentrations of *L. monocytogenes*. Most cases of individuals were exposed to food contaminated with 3.5 to 7.5 log CFU/serving of *L. monocytogenes*. In Europe, 90% of listeriosis was considered to be caused by consumption of RTE foods containing >2000 CFU/g of *L. monocytogenes* particularly in people over 64 years of age (European Food Safety Authority, 2016). These values were higher than the 100 cells/g of ICMSF. On the contrary, in the United States, a "zero-tolerance" is implemented for RTE products sold in their market regardless of its contamination level. Buchanan et al. (2017) added that the probability of illness depends on the strain of *L. monocytogenes* and the individual. Determining the role of food in the growth of *L. monocytogenes* and its effect on the microorganism's survivability are important.

Table 2
Growth kinetic parameters[1] of autochthonous spoilage microorganism in coconut water stored at different storage temperatures.

Storage temperatures (°C)	Pop_{init} (log CFU/mL)	t_{lag} (h)	K_G (log CFU/h)	Pop_{fin} (log CFU/mL)	R^2
4	3.2 ± 0.4^a	46.2 ± 26.9^a	0.031 ± 0.004^d	9.2 ± 0.2^a	0.93–0.99
17	2.8 ± 0.3^{ab}	0.0 ± 0.0^b	0.179 ± 0.020^c	9.0 ± 0.0^b	0.98–1.00
30	2.5 ± 0.2^b	0.0 ± 0.0^b	0.383 ± 0.012^b	8.9 ± 0.1^b	0.96–0.97
35	3.2 ± 0.1^a	3.4 ± 0.6^b	0.707 ± 0.087^a	7.6 ± 0.0^c	0.97–0.98

a,b,c… Values on the same column followed by the same letters are not significantly different ($P > 0.05$).

[1] Values are reported as averages of 4 readings obtained from 2 independently ran experiments.

The SRT values determined in the test conditions are presented in Fig. 2a and are compared with the microbiological shelf life (SL) of the commodity. The SL was determined to further determine the risk in the context of the marketability of the commodity. Results showed SRT < SL in 4 of the tested conditions. That is, the 2-log increase in the pathogen population that took place ahead of the end of shelf life conditions, which included both the recommended and abused storage for the commodity. It should be noted that in the abused conditions of 30 °C, which represent the vending of coconut water at ambient conditions, the SRT < SL were observed in the commodity with low contamination levels. In all the abuse storage condition, the SL were longer than the recommended 2–4 h storage for ready to eat commodities exposed to the temperature danger zone (FSANZ, 2019). Hence, in the absence of a mechanism that could determine the end SL, following such recommendation would have protected highly susceptible consumers from possible infections. These results further strengthen the bases for recommendations for good hygienic and manufacturing practices, especially for such a vulnerable commodity.

Furthermore, the very similar growth behavior of the pathogen at low- and high contamination levels is shown in the almost parallel curves in Fig. 2b. This resulted in a temperature coefficient (Q_{10}) values with <1.0 h difference. These observed behaviors are slightly different from those reported by Sinigaglia et al. (2006) for *L. monocytogenes* in coconut meat inoculated with low (Q_{10} = 1.85 to 2.11 days) and high levels (Q_{10} = 2.28 to 3.27 days) of the pathogens. The difference may be explained by variations in the strains used and the populations introduced to the food. The steeper slope reported by Sinigaglia et al. (2006) for the organism in coconut meat with high contamination may be attributed to the higher population (*ca.* 5.0 log CFU) introduced.

4. Conclusion

The study determined the growth kinetic parameters of *L. monocytogenes* in green coconut water to establish the Sanitary Risk Time for the food product stored at various temperatures (4, 17, 30, 35 °C) and subjected to low (~2.0 log CFU/mL) and high (~4.0 log CFU/mL)contamination levels. The growth of autochthonous spoilage microbiota in the different storage conditions were also determined to establish the microbiological shelf life of the commodity. Results showed that within the observation period, the pathogen grew in all tested conditions, except in the lowest test temperature at low contamination level. In some of the conditions tested, the Sanitary Risk

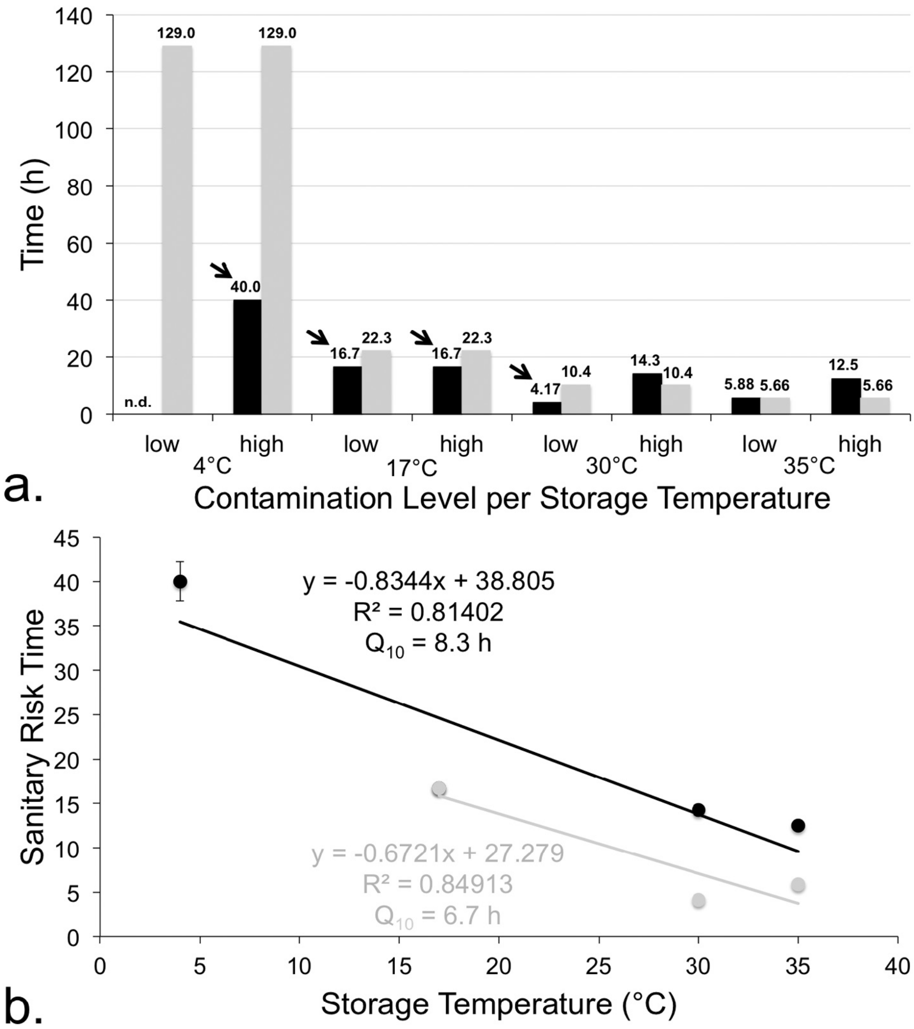

Fig. 2. (a) Comparisons of Sanitary Risk Times (SRTs, black bars) and microbial shelf life (SL, grey bars); and (b) quantification of temperature coefficient (Q_{10}) for SRTs at low (grey curve) and high (black curve) contamination levels. Arrows indicate conditions where SRT < SL, which could potentially result in infection.

Time took place earlier than the end of shelf life, emphasizing the importance of having good hygienic and manufacturing practices in place for such a vulnerable commodity.

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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