



Decimal reduction energies of UV-C-irradiated spoilage yeasts in coconut liquid endosperm

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ABSTRACT

The ultraviolet-C (UV-C) decimal reduction energy (D_{UV-C}) values of 17 spoilage yeasts and their composited inoculum were determined in coconut liquid endosperm (pH 5.26, 5.8 °Brix, 0.04% malic acid, 0.17% w/v insoluble solids). Growth kinetic parameters of all the test yeast strains were first established to standardize the growth stage of the cells prior to inactivation studies. Approximately 4.0 to 5.0 log CFU/mL cells in the mid-stationary growth phase (30.3 to 39.9 h, 25 °C) were suspended in 4 mL turbulent flowing juice and subjected to UV-C irradiation at a surface irradiance range of 3.42 to 4.99 mW/cm². Survivor populations after exposure to predetermined UV-C energy were enumerated, and were used to derive the D_{UV-C} values using the linear regression and Baranyi and Roberts (1994) model fitting. Results show that the yeast strains exhibited either log-linear or biphasic inactivation behavior with inactivation lag. The most UV-C resistant spoilage yeast was found to be *Cryptococcus albidus* (LJY1) with D_{UV-C} values of 122.72 and 214.89 mJ/cm² determined from linear regression and model-fitting, respectively. The least UV-C resistant was *Torulopsis delbrueckii* (LYJ5) with a D_{UV-C} of 17.34 (linear regression) and 17.35 mJ/cm² (model-fitting). The D_{UV-C} values determined from the model fitting were generally greater than those calculated from linear regression, although only those determined for *C. albidus* were significantly different. To the investigators' knowledge, this is the first report of the UV-C inactivation kinetic parameters of *Kluyveromyces marxianus*, *Trichosporon cutaneum*, *Pichia anomala*, and *Meyerozyma guilliermondii* and *C. albidus* in coconut liquid endosperm. The results of this study can be used in the establishment and validation of UV-C process schedules for coconut liquid endosperm and other similar commodities.

1. Introduction

High temperature food preservation technology is the most popular technique used for fruit and vegetable juices because it is simple and widely accessible to food processors (Ling et al., 2015; Petrucci et al., 2017). However, some of the desirable nutritional and sensory qualities of finished products are sensitive to heating (Choi and Nielsen, 2005; Esteve and Frigola, 2008; Marcel et al., 2014; Patras et al., 2010). One of the more commonly reported characteristics to be significantly affected by thermal processing is color (Chandran et al., 2014; Gabriel et al., 2017; Rattanathanalerk et al., 2005; Torres Gama and de Sylos, 2007) Furthermore, ascorbic acid content and antioxidant activity has been shown to be negatively affected by thermal processing (Bansal et al., 2015; Gabriel et al., 2015a, 2015b; Radziejewska-Kubzdela and

Biegańska-Marecik, 2015). Ultraviolet-C (UV-C) processing of fruit juices is a nonthermal alternative that could address these limitations of high temperature processing. UV-C technology entails lower financial costs compared to thermal processing and other novel processing technologies (Adzahan et al., 2011; Rodriguez-Gonzalez et al., 2015). UV-C food processing it does not result in the liberation of undesirable chemical residues and by-products that negatively affect finished product quality and safety (Guerrero-Beltran and Barbosa-Cánovas, 2004).

The United States Food and Drug Administration (USFDA) and United States Department of Agriculture (USDA) approved UV-C as a suitable processing technology for juice pasteurization (Federal Register, 2001). This technology harnesses the germicidal ultraviolet light region at 200–800 nm. (Falguera et al., 2011). The UV-C region can induce more mutagenic lesions to the DNA and results in the

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formation of products such as cyclobutane pyrimidine dimers (CPD), pyrimidine 6-4 pyrimidone photoproducts (6-4 PPs) and its Dewar isomers (Gayán et al., 2014; Sinha and Häder, 2002). These changes lead to the alteration of important DNA biochemistry that eventually results in cell inactivation (Bolton and Linden, 2003).

Since UV-C has been approved for pasteurization of fruit juices, published works commonly report the inactivation of disease-causing bacteria rather than spoilage-causing microorganisms (Fredericks et al., 2011; Gabriel, 2012; Gayán et al., 2014; Gouma et al., 2015; Guerrero-Beltran and Barbosa-Cánovas, 2004; Kaya et al., 2015; Quintero-Ramos et al., 2004). The dearth of information on the UV-C resistance of fruit juice spoilage organisms such as yeasts is a challenge worth addressing since yeasts were previously demonstrated to be more resistant towards UV-C than pathogenic bacteria (Gabriel, 2012). Furthermore, the diversity of yeasts species present in different fruit juices is another impetus for the establishment of their UV-C resistance in these commodities. Previous studies have enumerated yeasts including *Candida intermedia*, *Candida parapsilosis*, *Candida tropicalis*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Pichia anomala*, *Pichia fermentans*, *Saccharomyces cerevisiae*, *Torulaspota delbrueckii*, *Trichosporon mucoides*, *Meyerozyma guilliermondii*, *Kluyveromyces marxianus*, and *Cryptococcus albidus* in citrus juices, coconut liquid endosperm, cashew juice, black currant, and pineapple (Arias et al., 2002; Corte et al., 2015; Gabriel et al., 2018; Maciel et al., 2013; Senses-Ergul et al., 2006).

Yeasts can be introduced to the product, especially when the juice is manually extracted from the source such as that of coconuts. The inherent physicochemical properties and nutritional value, and the very manual process of extracting and preparing coconut beverages make coconut liquid endosperm beverages ideal growth media for spoilage- and disease-causing microorganisms (Gabriel and Colombo, 2016; Reddy et al., 2005; Rolle, 2007). Spoilage yeasts organisms of economic significance since these are responsible for the alteration and loss of quality attributes of fruit juices such as aroma, flavor, taste, color, and loss or presence of cloudiness (Bevilacqua et al., 2011). Spoilage of fruit juices due to yeasts is frequently characterized by the presence of carbon dioxide, alcohol and fermented flavor due to organic acids produced as well as occasional flocculation and pellicle formation (Aneja et al., 2014). Highly fermentative yeasts can cause explosion of plastic or glass bottles and splitting of can and cartons (Bevilacqua et al., 2011). This study therefore aimed to help address the dearth of information on the UV-C inactivation behavior and inactivation rates of spoilage yeasts isolates in a coconut liquid endosperm beverage suspending medium. Furthermore, the goal was to determine a UV-C resistant isolate that can be used as a reference organism for the establishment of UV-C process schedule for coconut liquid endosperm and similar commodities.

2. Materials and methods

2.1. Coconut liquid endosperm beverage

A commercially available coconut liquid endosperm (Nyogi Pure Coconut Water, My Philippines Lifestyle, Inc., Philippines) was used as the suspending medium for the inactivation studies. The Soluble solids (SS, °Brix) and pH were measured using a handheld refractometer (Atago, Tokyo, Japan) and pH meter (Eutech pH 700, Eutech Pte., Ltd., Singapore) respectively. Moreover, the titratable acidity (TA, % malic acid) was determined following the AOAC Method 942.15 (AOAC, 2000) using colorimetric titration with 0.1 N NaOH (RCI Labsan, Thailand) until phenolphthalein endpoint, indicated by a faint pink color which persisted for 30 s. The amount of suspended insoluble solids (IS) was determined by filtering 5 mL of coconut liquid endosperm in 0.47 µm pore-sized membrane filters (Advantech, Toyo Roshi Kaisha, Ltd. Japan). The filter paper with the residue was dried for 8–12 h in a desiccator until constant weight for three consecutive measurements. The dried residue represented the insoluble solids

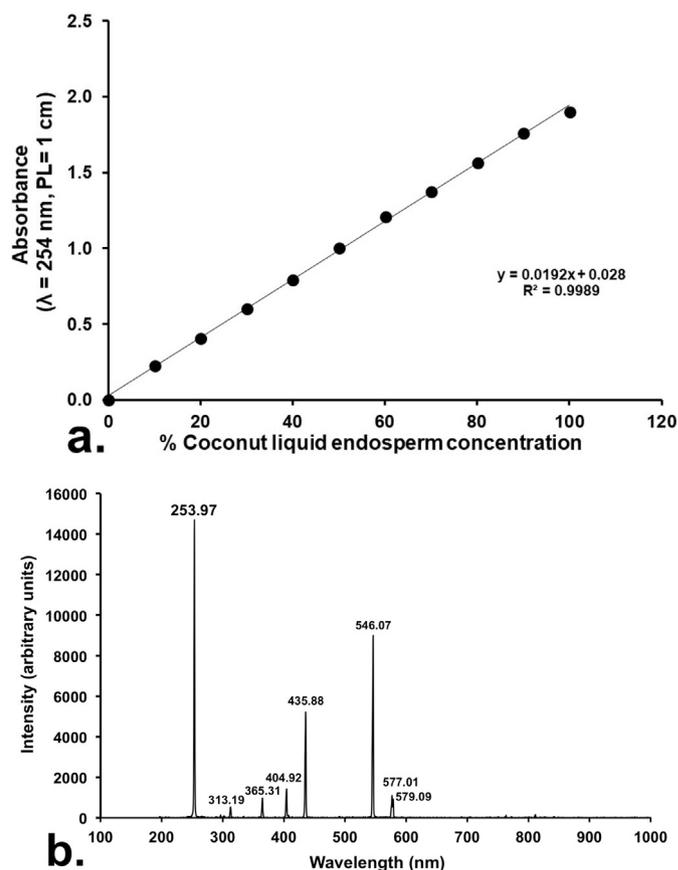


Fig. 1. Optical characteristics of the test coconut liquid endosperm and UV-C lamp. (a) A plot of coconut liquid endosperm concentration vs. absorbance for the determination of the percent solution extinction coefficient ($\epsilon_{\text{percent}}$, slope of the regressed line) from which the extinction coefficient was determined (ϵ value). (b) Emission spectra of the 15 W UV-C lamp source showing predominant emission wavelength at 254 nm.

fraction of the juice and the result was expressed as %wt/vol of sample.

The UV absorption coefficient (ϵ) was determined by measuring the absorbance of increasingly diluted beverage samples at 254 nm at 1 cm path length using a UV/Vis double beam spectrophotometer (Unico SQ-4802, United Products & Instruments, Inc., New Jersey, USA). The ϵ was calculated from the slope of the linear absorption curve versus the diluted concentration of the juice (Fig. 1a). Prior to being used, the coconut liquid endosperm samples were analyzed for background microflora through spread plating 0.1 mL sample on appropriate growth media. The aerobic bacteria and yeast and mold counts were detected below detection limit ($< 10 \log \text{CFU/mL}$), hence removal of background microflora was deemed not necessary.

2.2. Test spoilage yeast and culture maintenance

The study used 17 spoilage yeasts strains isolated from spoiled food products (Table 1). The tested yeast strains included 3 isolates of *Candida* spp. and *Pichia* spp.; 2 isolates of *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, and *Torulaspota delbrueckii*; and 1 strain each of *Clavispora lusitaniae*, *Cryptococcus albidus*, *Kluyveromyces marxianus*, *Meyerozyma guilliermondii* and *Trichosporon cutaneum*. The yeasts were subjected into a series of activation, enrichment, and purification steps prior to use. Activation was done by aseptically transferring a loopful from the stock slant culture to a laboratory-compounded Yeast Extract Peptone Glucose Broth (YPGB) composed of 0.5% yeast extract (Becton Dickinson and Company, France), 0.1% peptone (Becton Dickinson and Company, France), and 2.0% glucose (Sigma-Aldrich, Japan). The

Table 1
Spoilage yeast isolates subjected to UV-C inactivation studies.

Yeast isolates	Isolate Codes	Origins	Culture collections
<i>Candida parapsilosis</i>	(LUSC1)	Spoiled <i>calamansi</i> juice drink	Laboratory of Food Microbiology and Hygiene, Department of Food Science and Nutrition, University of the Philippines Diliman (LFMH-UPD)
<i>Candida pseudointermedia</i>	(LJY2)	Spoiled ready to eat meal	Laboratory of Food Microbiology and Hygiene, Graduate School of Biosphere Science, Hiroshima University (LFMH-HU)
<i>Candida tropicalis</i>	(BIOTECH 2085)	Fermented fish	Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology, University of the Philippines-Los Baños (BIOTECH)
<i>Clavispora lusitanae</i>	(BFE-36)	Spoiled processed fruit product	Research and Development Center of the Aohata Corporation, Takehara, Hiroshima, Japan (Aohata Corp.)
<i>Cryptococcus albidus</i>	(LJY1)	Spoiled ready to eat meal	LFMH-HU
<i>Debaryomyces hansenii</i>	(BFE-34)	Spoiled processed fruit product	Aohata Corp.
<i>Debaryomyces hansenii</i>	(BIOTECH 2204)	Not reported	BIOTECH
<i>Kluyveromyces marxianus</i>	(BIOTECH 2223)	Not reported	BIOTECH
<i>Meyerozyma guilliermondii</i>	(LJY3)	Spoiled ready to eat meal	LFMH-HU
<i>Pichia anomala</i>	(BIOTECH 2205)	Not reported	BIOTECH
<i>Pichia fermentans</i>	(BFE-38)	Spoiled ready to eat meal	LFMH-HU
<i>Pichia fermentans</i>	(LJY6)	Spoiled processed fruit product	Aohata Corp.
<i>Saccharomyces cerevisiae</i>	(BFE-39)	Spoiled processed fruit product	Aohata Corp.
<i>Saccharomyces cerevisiae</i>	(BIOTECH-2019)	Alcoholic beverage	BIOTECH
<i>Torulasporea delbrueckii</i>	(BFE-37)	Spoiled ready to eat meal	LFMH-HU
<i>Torulasporea delbrueckii</i>	(LJY5)	Spoiled processed fruit product	Aohata Corp.
<i>Trichosporon cutaneum</i>	(BIOTECH-2089)	Fermented fish	BIOTECH

inoculated YPGB tubes were then incubated at 25 °C for 48 h. Subsequently, the activated yeast culture was enriched by transferring a loopful into a new YPGB tube, and subjected to another 48-h incubation at 25 °C. The enriched culture was then streaked onto Potato Dextrose Agar, incubated at 25 °C for 48 h (Nissui Pharmaceutical Co. Ltd., Japan) and stored at 4 °C until further use. Each of the yeast strains was subjected to the same 2-passage culture and slant streaking protocol every 14 d to maintain culture age throughout the growth modelling and UV-C inactivation studies.

2.3. Growth kinetic parameters of spoilage yeasts

The growth kinetic parameters of all yeast isolates were determined to control the possible effect of growth phase on the UV-C resistance of the cells. Cells from refrigerated slants were subjected to the previously described 2-passage culture protocols after which, a 0.4 mL aliquot was introduced to 49.6 mL YPGB. The culture broth was then incubated at 25 °C for 48 h. An aliquot of 1.0 mL was obtained at 0, 1, 2, 4, 8, 12, 24, 30, 36, and 48 h-intervals during incubation. Withdrawn samples were then subjected to serial 10-fold dilutions with sterile peptone water (Becton Dickinson and Company, France) and surface-plated onto pre-solidified Potato Dextrose Agar (PDA, Nissui Pharmaceutical Co. Ltd., Japan) acidified to pH 3.5 with 10% tartaric acid (Nacalai Tesque Inc., Kyoto, Japan). The plates were then incubated at 25 °C for 48 h prior to colony enumeration. The microbial growth populations of the yeasts were expressed as log CFU/mL. The growth kinetic parameters determined included the lag time (t_{lag}), growth rate (k_G), final population (Pop_{fin}), and midpoint of stationary phase (MSP). The midpoint of stationary phase was interpolated from the growth curve and determined as the midpoint between the start of the stationary phase and the last incubation time (48 h). The characterization of these growth kinetic parameters was done using the MS Excel add-in, Dynamic Modelling Fit (DMFit) Version 3.0 (Quadram Research Bioscience UK) based on the work of Baranyi and Roberts (1994).

2.4. Coconut liquid endospore inoculation and UV-C inactivation studies

Cells from refrigerated slants were subjected to the previously described 2-passage culture protocol with modification. On the second

passage, the cell suspension in YPGB was only incubated at 25 °C until the MSP. Cells were harvested by spinning 1.0 mL of the growth suspension on a bench top centrifuge (Kubota, KM-15200, Tokyo, Japan) at 8000 rpm for 10 min. The supernatant liquid was then decanted while the cell pellets were resuspended in 1.0 mL coconut liquid endospore and allowed to acclimatize for 10 min prior to UV-C inactivation studies.

Prior to UV-C irradiation, 0.04 mL aliquot of previously acclimatized test organism was diluted with 3.96 mL of coconut liquid endospore on 35-mm sterile plastic Petri dishes. This was done to introduce an initial inoculum population of approximately 4.0–5.0 log CFU/mL. The Petri dish with the inoculated juice had a 7-mm liquid sample thickness while containing a 1.25-cm magnetic spin bar. The inoculated juice was then subjected to UV-C radiation by placing each plate on a magnetic stirring plate under a fabricated UV-C box with three 15-W mercury vapor lamps (Sankyo Denki, Japan) at a lamp-to-sample surface distance of 100 mm. A sample UV-C lamp used in this study was subjected to optical emission spectroscopy that characterized predominant radiation emission of 254 nm at the same treatment distance. Measurements were done using a Spectrometer (Ocean optics, Inc., FL., USA) with a dispersion of 0.2467 nm per pixel and an optical resolution of 1.0855 nm in the range of 200–1100 nm (Fig. 1b).

The inoculated juice was stirred at maximum rotational speed of 1500 rpm while being exposed to UV radiation at room temperature (25 °C) at different time intervals between 0 and 90 s to allow even exposure. Simultaneous radiometric readings (UVX Radiometer, UVP, Upland, California) obtained show that the liquid endospore surface received a UV-C irradiance range of 3.42 to 4.99 mW/cm². Thus by the end of the 90 s irradiation, the liquid endospore surface received UV-C energy of 307.8 to 449.1 mJ/cm². To determine survivor populations, the treated juices were then subjected to serial 10-fold dilutions with sterile peptone water and surface-plated onto pre-solidified, acidified PDA. Colonies were enumerated after incubation at 25 °C for 48 h. The enumerated populations were expressed as log CFU/mL. The UV-C inactivation was conducted with 2 independent external runs with 2 internal replicates each. In addition, UV-C inactivation studies on composed inoculum containing all 17 yeast strain were also investigated. The composite inoculum was prepared by subjecting the yeast strains to

previously described modified 2-passage culture protocol. An aliquot of 1.00 mL was obtained from each of the enriched yeast strains and combined by mixing in a sterile flask; after which 1 mL of the composed mixture was obtained and subjected to previously described cell harvesting, acclimatization and inactivation, and survivor enumeration protocols.

2.5. UV-C inactivation behaviors and inactivation parameters

The UV-C inactivation kinetic parameters curves and inactivation behavior of the spoilage yeasts were characterized using the linear regression function of the Microsoft Excel, and through model fitting using the model established by Baranyi and Roberts (1994) using DMFiT 3.0 MS Excel add-in. The linear regression model was able to calculate the equation of the best fit straight line from the inactivation data. The negative inverse of the slope from the equation was then used to determine the decimal reduction time (D value) or the UV-C exposure time (s) necessary to induce a 90% (1 log) reduction in the yeast population at fixed irradiance. The decimal reduction energies (D_{UV-C}) values were also determined, which corresponded to the amount of UV-C energy dose necessary to inactivate the target organism by 1 log cycle at a fixed irradiance. The D_{UV-C} values were obtained by multiplying the measured UV-C irradiance by the D value. The fit of the data with respect to the determined straight line was measured by R^2 . Fitting into the Baranyi and Roberts (1994) model was able to characterize non-logarithmic-linear inactivation behavior, and was able to determine when possible, the inactivation lag time (t_{lag}), and the death rate (k_D), of each test organism and their composite. The D value was calculated by obtaining the negative inverse of the k_D . For biphasic inactivation behavior with a lag time, the D' was determined as the sum of t_{lag} and k_D^{-1} . The D_{UV-C} values were similarly determined following previously described protocol.

2.6. Statistical analysis

Data obtained from independently 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). Duncan's Multiple Range Test was used for post-hoc determinations of significant differences at 95% level of significance ($P < 0.05$).

3. Results and discussion

3.1. Physicochemical properties of coconut liquid endosperm beverage

The test coconut liquid endosperm had a pH of 5.26, which was similar to previously reported pH of coconut water (5.06) (Gabriel and Colombo, 2016), and within the range (4.62–5.43) previously reported for coconut liquid endosperm sampled from several provinces of the Philippines (Gabriel and Arellano, 2014). This pH was also close to those of low acid juices such as watermelon (5.19), and carrot juice (5.75) (Koutchma, 2009; Quek et al., 2007). The SS of the test beverage was 5.80 °Brix, which is slightly lower than the previously reported value of 6.10 °Brix (Gabriel and Colombo, 2016), but within the range reported by Gabriel and Arellano (2014) of 4.80–5.83. As expected, the test beverage contained very small amount of insoluble suspended solids (0.17%wt/vol). The TA determined for the tested beverage was 0.04%malic acid, which was lower than the range reported for coconut liquid endosperm in the Philippines (0.05–1.2%). Furthermore, an ϵ value of 1.92 cm^{-1} was determined for the test beverage, which was 10-fold higher than that previously reported by Gabriel and Colombo (2016). It should be noted that this current study used a commercially available beverage, while the reported value was obtained from freshly extracted coconut liquid endosperm.

Moreover, the ϵ of the beverage was lower than other semi-

transparent beverages such as clear apple juice (25.9 cm^{-1}), beer (16.0 cm^{-1}), sherry wine (9.0 cm^{-1}), liquid sucrose (4.5 cm^{-1}), passion fruit juice (11.7 cm^{-1}) and watermelon juice (23.6 cm^{-1}) (Koutchma, 2009; Koutchma et al., 2007). The differences in the physicochemical properties reflect the relatively simple composition of coconut liquid endosperm compared to other liquid food systems. The sterile juice is reported to contain water, soluble sugar, proteins, vitamins, minerals, oil and phytohormones (Yong et al., 2009). It is expected that these will shift higher as the coconut fruit develops in maturity (Prades et al., 2012). Several studies have shown the attenuation of UV-C on the inactivation of microorganisms because of varying physicochemical properties of suspending medium (Estilo and Gabriel, 2017; Gabriel and Marquez, 2017). Moreover, the study of Koutchma and Parisi (2004) showed that increasing soluble solids lead to an increase in the absorption coefficient due to the light scattering effects of suspended particles.

3.2. Growth curves and growth kinetic parameters

The growth kinetics of the tested yeast were established to achieve uniformity in the growth stage of the spoilage yeasts and to minimize the influence of growth phase on the resistance of cells towards inactivating agent (Child et al., 2002; Gabriel et al., 2016; Nair and Finkel, 2004). The growth curves of the tested spoilage yeasts are presented in Fig. 2, while the growth kinetic parameters are summarized in Table 2. Growth lag was observed only for 3 yeast strains, which ranged from 1.69 h (*S. cerevisiae* BFE-39) to 3.44 h (*C. lusitaniae* BFE-36). However, despite having a lag phase *C. lusitaniae* (BFE-36) exhibited the fastest growth rate (0.61 h log CFU/h) among the spoilage yeasts ($P < 0.05$) This growth rate was greater than the slowest growth rate of *T. cutaneum* (BIOTECH2089) by 6 folds. Gabriel (2012) similarly reported growth lag in *S. cerevisiae* BFE-39, *D. hansenii*, *T. delbrueckii*, and *P. fermentans* in apple juice. The observed disparity in microbial growth behavior may be attributed to the differences in the physicochemical properties of the growth medium used. While apple juice can support the growth of yeasts, its inherent acidity, sugar content, and other components must have caused the slight delay in cell division. Yates and Smotzer (2007) explained that lag times represent the time for cells to adjust and resume physiological functions that are necessary for survival in the growth medium. These physiological functions precede the initiation of cellular multiplication.

The stationary phase of the selected spoilage yeasts occurred earlier than those reported in literature, where it was usually reached after several days of incubation (Herman, 2002; Uppuluri and Chaffin, 2007; Werner-Washburne et al., 1993). However, the results were similar to those obtained by Gabriel (2012). The differences in the onset of stationary phase can be attributed to the differences in the nutrients available in the growth medium. In previous reports, the concentrations of glucose in the growth medium were higher, thereby allowing the yeast growth rate to increase for a longer period. Inflection of the growth curve to stationary phase indicates that nutrients are already exhausted (Herman, 2002; Jay et al., 2006). During this phase, the growth rate is at its minimum, bringing physiological changes in yeasts including the accumulation of glycogen, increased resistance to a variety of environmental stresses, heat shock, a thickening of the cell wall, and the increased ability to survive extended periods of starvation (Herman, 2002). The spread of the MSP values determined from the 17 tested yeast isolates was not high. The MSP ranged from 31.0 h for *C. lusitaniae* (BFE-36) to 39.0 h for *C. albidus* (LJY1).

3.3. UV-C inactivation behaviors of spoilage yeasts

The various spoilage yeasts in their MSP growth phase, suspended in UV-C-irradiated coconut liquid endosperm beverage exhibited both log-linear inactivation behaviors and biphasic inactivation behavior, depending on the method used to characterize the inactivation patterns.

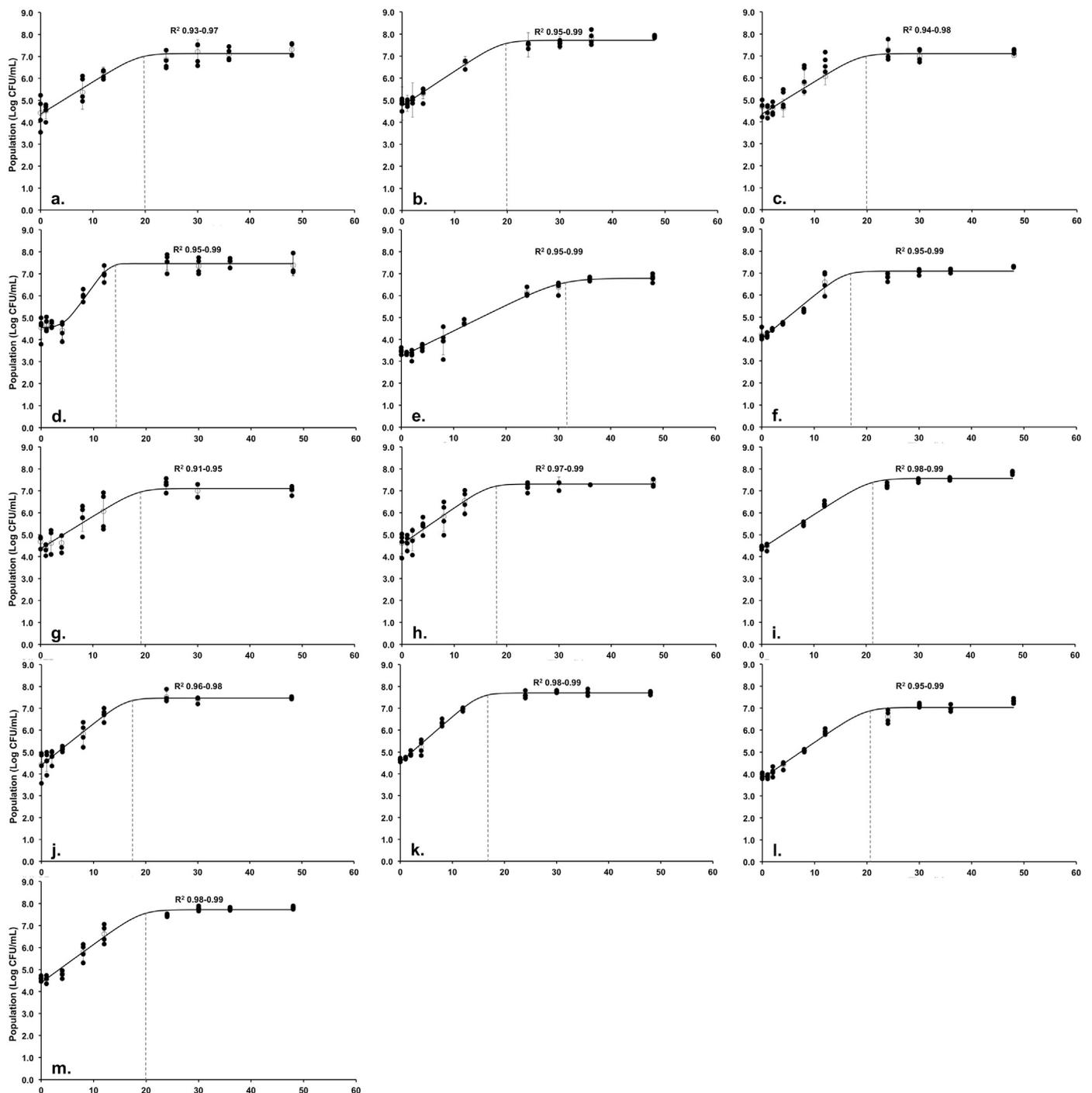


Fig. 2. Growth curves of test yeast isolates in YPG broth at 25 °C. (a) *C. parapsilosis* (LUSC1), (b) *C. pseudointermedia* (LJY2), (c) *C. tropicalis* (BIOTECH 2085), (d) *C. lusitanae* (BFE-36), (e) *C. albidus* (LJY1), (f) *D. hansenii* (BFE-34), (g) *D. hansenii* (BIOTECH 2204), (h) *K. marxianus* (BIOTECH 2223), (i) *M. guilliermondii* (LJY3), (j) *P. anomala* (BIOTECH 2205), (k) *P. fermentans* (BFE-38), (l) *P. fermentans* (LJY3), (m) *S. cerevisiae* (BFE-39), (n) *S. cerevisiae* (BIOTECH 2019), (o) *T. delbrueckii* (BFE-37), (p) *T. delbrueckii* (LJY5), and (q) *T. cutaneum* (BIOTECH 2089). Black markers represent population obtained in duplicate runs, with each run having two internal replicates. White markers represent average population values obtained per sampling time. Dotted vertical lines denote the microbial growth inflection to stationary phase.

The inactivation plots of the spoilage yeasts are presented in Fig. 3. Log-linear inactivation behavior determined by liner regression resulted in curves with $R^2 > 0.91$. Fitting the same inactivation data to the Baranyi and Roberts (1994) model resulted in biphasic inactivation behavior in some of the yeast isolates, specifically *C. parapsilosis* (LUSC1), *C. tropicalis* (BIOTECH 2085), *C. lusitanae* (BFE-36), *C. albidus* (LJY1), *D. hansenii* (BIOTECH 2204), *K. marxianus* (BIOTECH 2223) *M. guilliermondii* (LJY3), *P. anomala* (BIOTECH 2205), *P. fermentans* (BFE-

38) and *T. cutaneum* (BIOTECH 2089). This biphasic inactivation behavior is characterized by a curve with downward concavity that coincides with inactivation lag where minimal or no inactivation takes place. This inactivation lag is followed by a fast log-linear inactivation behavior. The longest inactivation lag time was observed for *C. albidus* (LJY1) at 25.35 s followed by *T. cutaneum* (BIOTECH 2089) at 15.05 s, while the shortest was that of *P. anomala* (BIOTECH 2205) at 0.56 s. The Baranyi and Roberts (1994) model was able to fit the inactivation

Table 2
Growth kinetic parameters¹ of test yeast isolates in Yeast Extract Peptone Glucose Broth.

Yeast isolates	Lag (T _{lag} , h)	Rate (K _G , log CFU/h)	Pop _{fin} (log CFU/mL)	MSP (h) ²	R ²
<i>C. parapsilosis</i> (LUSC1)	2.26 ± 2.65 ^a	0.14 ± 0.04 ^b	7.15 ± 0.37 ^{cde}	35.1 ± 2.0 ^{bcd}	0.93–0.97
<i>C. pseudointermedia</i> (LJY2)	0.00 ± 0.00 ^b	0.16 ± 0.02 ^b	7.72 ± 0.15 ^a	33.9 ± 1.3 ^{bcde}	0.95–0.99
<i>C. tropicalis</i> (BIOTECH 2085)	0.00 ± 0.00 ^b	0.20 ± 0.02 ^b	7.13 ± 0.29 ^{cde}	31.6 ± 1.3 ^e	0.94–0.98
<i>C. lusitanae</i> (BFE-36)	3.44 ± 2.99 ^a	0.61 ± 0.67 ^a	7.43 ± 0.32 ^{abc}	31.0 ± 1.8 ^e	0.95–0.99
<i>C. albidus</i> (LJY1)	0.00 ± 0.00 ^b	0.12 ± 0.01 ^b	6.79 ± 0.13 ^{ef}	39.9 ± 1.8 ^e	0.95–0.99
<i>D. hansenii</i> (BFE-34)	0.00 ± 0.00 ^b	0.19 ± 0.04 ^b	7.10 ± 0.05 ^{cde}	32.8 ± 1.7 ^{cde}	0.95–0.99
<i>D. hansenii</i> (BIOTECH 2204)	0.00 ± 0.00 ^b	0.16 ± 0.03 ^b	7.05 ± 0.29 ^{de}	33.6 ± 2.0 ^{bcde}	0.91–0.95
<i>K. marxianus</i> (BIOTECH 2223)	0.00 ± 0.00 ^b	0.16 ± 0.02 ^b	7.33 ± 0.16 ^{cde}	33.5 ± 2.1 ^{bcde}	0.97–0.99
<i>M. guilliermondii</i> (LJY3)	0.00 ± 0.00 ^b	0.16 ± 0.02 ^b	7.58 ± 0.08 ^{ab}	34.9 ± 0.9 ^{bcd}	0.98–0.99
<i>P. anomala</i> (BIOTECH 2205)	0.00 ± 0.00 ^b	0.19 ± 0.02 ^b	7.46 ± 0.06 ^{abc}	33.0 ± 1.1 ^{bcde}	0.96–0.98
<i>P. fermentans</i> (BFE-38)	0.00 ± 0.00 ^b	0.21 ± 0.01 ^b	7.70 ± 0.07 ^a	32.3 ± 0.3 ^{de}	0.98–0.99
<i>P. fermentans</i> (LJY6)	0.00 ± 0.00 ^b	0.15 ± 0.03 ^b	7.08 ± 0.11 ^{cde}	35.8 ± 1.7 ^{bc}	0.95–1.00
<i>S. cerevisiae</i> (BFE-39)	1.69 ± 3.38 ^a	0.24 ± 0.15 ^b	7.73 ± 0.04 ^a	33.8 ± 2.2 ^{bcde}	0.98–0.99
<i>S. cerevisiae</i> (BIOTECH-2019)	0.00 ± 0.00 ^b	0.19 ± 0.03 ^b	7.21 ± 0.09 ^{cd}	32.0 ± 2.0 ^{de}	0.96–0.98
<i>T. delbrueckii</i> (BFE-37)	0.00 ± 0.00 ^b	0.18 ± 0.00 ^b	7.59 ± 0.06 ^{ab}	32.5 ± 0.4 ^{de}	0.97–0.98
<i>T. delbrueckii</i> (LJY5)	0.00 ± 0.00 ^b	0.14 ± 0.03 ^b	7.71 ± 0.14 ^a	35.8 ± 3.0 ^{bc}	0.91–1.00
<i>T. cutaneum</i> (BIOTECH-2089)	0.00 ± 0.00 ^b	0.11 ± 0.02 ^b	6.67 ± 0.60 ^f	36.1 ± 3.4 ^b	0.96–0.99

^{a, b, c...} Values on the same column followed by the same superscript are not significantly different ($P > 0.05$).

¹ Averages of 4 values ± SD obtained from 2 independent runs. Growth kinetic parameters of the yeast isolates were determined by fitting into the Baranyi and Roberts (1994) model using the DMFit MS Excel add in (Quadram Research Bioscience, UK).

² Mid-stationary phase was determined per yeast isolate as midpoint between the start of the stationary phase and the last incubation time (48 h).

data of all the tested yeast strains including the composited strains with an R² range of 0.91–1.00.

Differences between the inactivation behavior of spoilage yeasts in literature and the results obtained in this study were observed. In a previous study reported by Gabriel (2012) on the ultraviolet inactivation of yeasts in apple juice, a biphasic inactivation behavior was observed for *T. delbrueckii* BFE-37, while a log-linear inactivation behavior was observed for *C. lusitanae*, *P. fermentans* and *S. cerevisiae*. A biphasic inactivation behavior was also reported for *Dekkera bruxellensis*, *Dekkera anomala*, *Saccharomyces bayanus*, *S. cerevisiae*, and *Zygosaccharomyces bailii* in McIlvane buffer (Gouma et al., 2015). The occurrence of biphasic inactivation behavior characterized by the initial inactivation lag was previously explained to be due to presence of clumps of cells, accumulation of cumulative physiological injury prior to inactivation, and re-synthesis of critical cellular components necessary for survival (Bevilacqua et al., 2015; Geeraerd et al., 2000; Xiong et al., 1999).

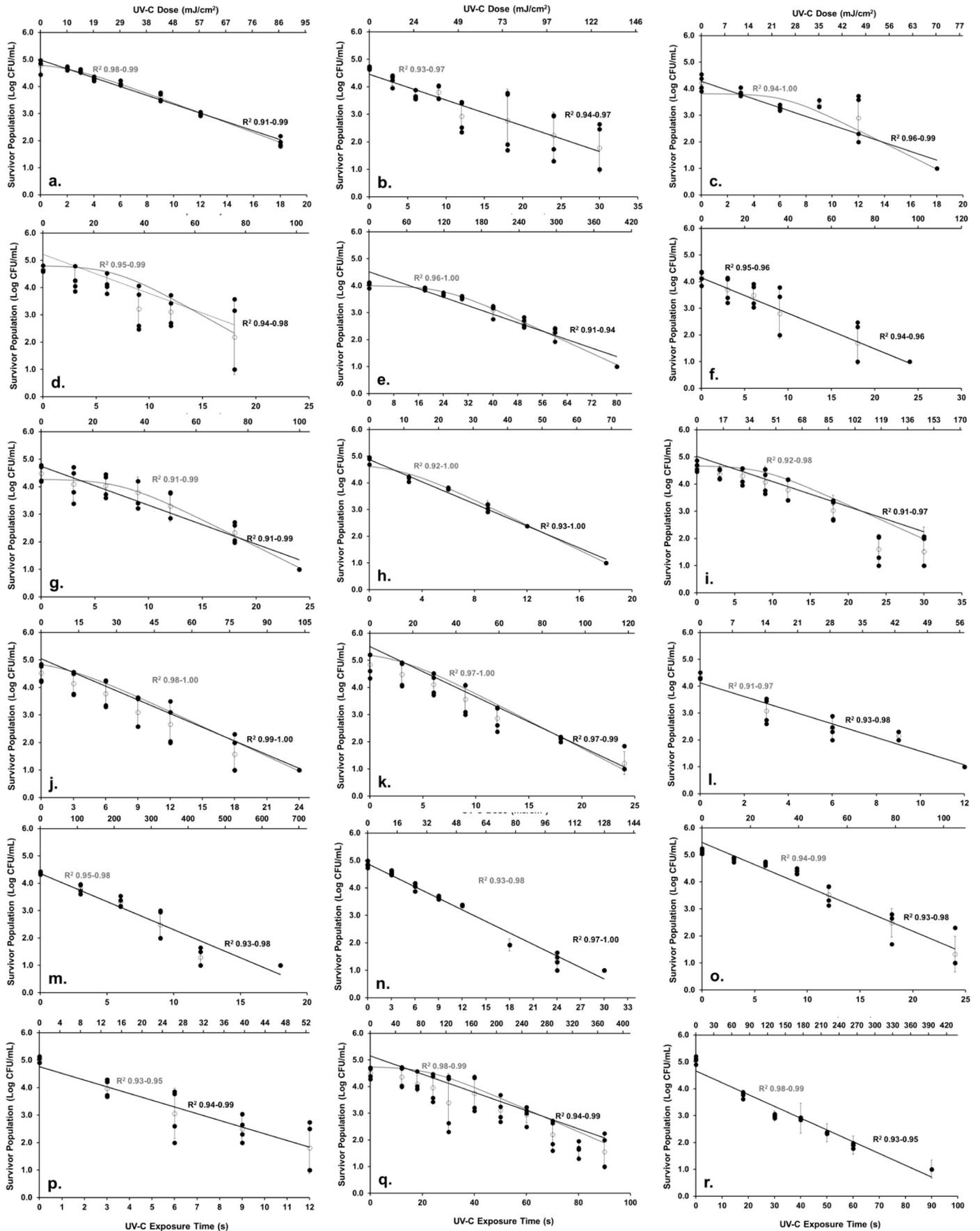
3.4. Inactivation kinetic parameters of spoilage yeasts

The inactivation kinetic parameters of the spoilage yeasts determined in from the linear regression and Baranyi and Roberts (1994) fitting are presented in Table 3 and Fig. 4. From the inactivation kinetic parameters determined by the linear regression method, *C. albidus* (LJY1) exhibited the highest UV-C resistance with a D_{UV-C} value of 122.72 mJ/cm² (D = 24.82 s), which was followed by *T. cutaneum* (BIOTECH-2089), with a significantly ($P > 0.05$) lower D_{UV-C} value of 110.01 mJ/cm² (D = 26.74 s). The composited yeast isolates had the 3rd highest resistance with a D_{UV-C} value of 99.96 mJ/cm² (D = 22.76 s). It is worthy to note that the trend observed for D_{UV-C} values was not the same as that for D values. This is because the D_{UV-C} value is dependent on the UV-C irradiance measured during specific inactivation runs. Hence, the D_{UV-C} value is a better metric for UV-C resistance rather than the D value. In the linear regression method, strains from the same genus and species were found to have significantly ($P < 0.05$) varying D_{UV-C} values. For test strains under the genus *Candida*, *C. pseudointermedia* (LJY2) was found to be significantly more resistant than the other *Candida* spp. by at least 40.67%. It should also be noted that the test *C. pseudointermedia* had the greatest variability in the calculated inactivation kinetic parameters using both the linear regression and model-fitting methods. Since a uniform set of protocols were applied in the propagation, inoculation, challenge study, and survivor enumeration for all test yeast strains, the study can only

surmise that the observed variation may be attributed to implicit characteristics of this particular strain that was not addressed in the methodology. Since culture age was controlled in all test strains, possible contributors to the observed variation include cohesion of cells into clumps that protected cells within the aggregates; or adhesion of cells on suspended particles or container walls that diminished the biocidal efficacy of UV-C.

The 2 *P. fermentans* had D_{UV-C} values that were significantly different, with *P. fermentans* (BFE-38) 43.96% more resistant than *P. fermentans* (LJY6). These differences were however, not observed when the inactivation kinetic parameters were determined using the Baranyi and Roberts (1994) model fitting. The tested *C. albidus* (LJY1) was similarly the most UV-C resistant when the inactivation data were fitted to the Baranyi and Roberts (1994) model with a D_{UV-C} value of 214.89 mJ/cm² (D' = D + lag = 43.46 s). This was followed by *T. cutaneum* (BIOTECH-2089) with a significantly lower D_{UV-C} value of 159.91 mJ/cm² (D' = 38.54 s). The composited yeast strains were also determined to have the 3rd highest resistance towards UV-C with a D_{UV-C} value of 99.97 mJ/cm² (D' = 22.76 s). In both methods of inactivation kinetic parameter calculations, the D_{UV-C} and D values of the rest of the yeast isolates did not spread too widely as summarized in Table 3.

The inactivation lag times of the different spoilage yeasts were mostly shorter than those observed by Gouma et al. (2015) in the inactivation of *Saccharomyces bayanus*, *S. cerevisiae*, and *Zygosaccharomyces bailii*, inactivation lag times ranging from 0.63 to 1.21 min. Moreover, in the study done by Gabriel (2012), the lag times of *T. delbrueckii* during UV-C inactivation in apple juice was longer at 1.64 to 2.47 min. This could be due to the differences in the inactivation set ups used between these reported studies. The UV-C resistance of spoilage yeasts is relatively higher than vegetative bacterial cells, but is usually lower than that of algae, bacterial spores, and molds (Guerrero-Beltran and Barbosa-Cánovas, 2004). The D_{UV-C} values of the spoilage yeasts established in this study were generally higher than those reported for *Escherichia coli* O157:H7 (11.72 mJ/cm²), *Salmonella enterica* (9.59 mJ/cm²), *Listeria monocytogenes* (8.28 mJ/cm²) and the coconut water spoilage bacteria *Staphylococcus epidermis* (11.72 mJ/cm²), *Kluyvera* sp. (7.63 mJ/cm²) and *Klebsiella* sp. (14.56 mJ/cm²) (Gabriel and Colombo, 2016). Aside from the variations in the characteristics of the structures that influence the UV-C resistance of cells, suspending medium intrinsic properties similarly affect UV-C resistance of microorganisms. The *C. parapsilosis* strain determined to be least resistant in orange juice in this current study was previously determined resistant compared to another



(caption on next page)

Fig. 3. UV-C inactivation curves of test yeast species in coconut liquid endosperm. (a) *C. parapsilosis* (LUSC1), (b) *C. pseudointermedia* (LJY2), (c) *C. tropicalis* (BIOTECH 2085), (d) *C. lusitanae* (BFE-36), (e) *C. albidus* (LJY1), (f) *D. hansenii* (BFE-34), (g) *D. hansenii* (BIOTECH 2204), (h) *K. marxianus* (BIOTECH 2223), (i) *M. guilliermondii* (LJY3), (j) *P. anomala* (BIOTECH 2205), (k) *P. fermentans* (BFE-38), (l) *P. fermentans* (LJY3), (m) *S. cerevisiae* (BFE-39), (n) *S. cerevisiae* (BIOTECH 2019), (o) *T. delbrueckii* (BFE-37), (p) *T. delbrueckii* (LJY5), (q) *T. cutaneum* (BIOTECH 2089), and (r) composite of all 17 isolates. Black markers represent population obtained in duplicate runs, with each run having two internal replicates. White markers represent average population values obtained per sampling time. Black curves represent linear regression model fit. Grey curves represent fit in the Baranyi and Roberts (1994) model.

C. parapsilosis isolate when suspended in *calamansi* juice drink ($\epsilon = 4.83 \text{ cm}^{-1}$) with a D_{UV-C} of 183.85 mJ/cm^2 (Gabriel et al., 2018). Moreover, the inactivation energies of the tested yeasts were lower compared to the inactivation of *S. cerevisiae* strains and natural yeast strains in freshly squeezed turbid grape juice (284.24 mJ/cm^2), pasteurized clear white grape juice (136.08 mJ/cm^2) and grape fruit juice (14 mJ/cm^2) (Geveke and Torres, 2012; Kaya and Unluturk, 2016).

To the investigators' knowledge this is the first study to report the UV-C inactivation kinetic parameters and behaviors of *K. marxianus*, *T. cutaneum*, *P. anomala*, *C. albidus*, and *M. guilliermondii* in coconut liquid endosperm. This study is also one of the few works that compared the UV-C resistance of several spoilage yeast strains to more accurately screen and select an appropriate reference organism for the establishment of a UV-C process schedule for fruit juices. In this study, the tested *C. albidus* was identified as possible reference organism. Visnhiac (1995) described *C. albidus* as an obligately aerobic, encapsulated, and the most common yeast species consistently associated with soil. Its ubiquity therefore makes it an organism of significant economic impact, as it can contaminate and eventually spoil raw materials and finished products. In fact, Stratford (2006) reported the presence of *C. albidus* and *C. diffluens* in the yeast flora of European and Asian soft drink factory, respectively. Davenport (1980) reported a minimum growth temperature of *C. albidus* at -12°C , and Stratford (2006) enumerated *C. albidus* and *C. magnus* as capable of inducing food and beverage spoilage at refrigerated conditions.

Table 3

Inactivation kinetic parameters of spoilage yeasts in coconut liquid endosperm.

Yeast isolates	Inactivation kinetic parameters calculated per method ²							
	Linear regression			Baranyi and Roberts (1994) model fitting				
	D (s) ³	D_{UV-C} (mJ/cm ²)	R ² range	Lag (s)	D (s)	D' (s) ⁴	D_{UV-C} ¹ (mJ/cm ²) ⁵	R ² range
<i>C. parapsilosis</i> (LUSC1)	6.19 ± 0.36 ^{fg}	29.60 ± 2.43 ^{efg}	0.91–0.99	1.81 ± 2.10 ^c	5.58 ± 0.68 ^{de}	7.39 ± 1.57 ^{cde}	35.30 ± 7.51 ^{def}	0.98–0.99
<i>C. pseudointermedia</i> (LJY2)	15.36 ± 11.49 ^d	49.89 ± 23.65 ^d	0.94–0.97	0.00 ± 0.00 ^c	11.06 ± 4.54 ^c	11.06 ± 4.54 ^c	48.34 ± 25.68 ^{de}	0.93–0.97
<i>C. tropicalis</i> (BIOTECH 2085)	6.86 ± 1.15 ^{fg}	26.78 ± 5.44 ^{efg}	0.96–0.99	1.59 ± 3.18 ^c	6.37 ± 1.82 ^{de}	7.96 ± 1.96 ^{cde}	30.91 ± 7.38 ^{def}	0.94–1.00
<i>C. lusitanae</i> (BFE-36)	5.72 ± 0.77 ^{ghi}	24.08 ± 4.16 ^{efg}	0.94–0.98	1.69 ± 3.38 ^c	5.35 ± 0.91 ^{de}	7.04 ± 2.95 ^{cde}	29.79 ± 13.45 ^{def}	0.95–0.99
<i>C. albidus</i> (LJY1)	24.82 ± 2.02 ^b	122.72 ± 10.18 ^a	0.91–0.94	25.35 ± 3.35 ^a	18.10 ± 2.21 ^b	43.46 ± 3.18 ^a	214.89 ± 15.55 ^a	0.96–1.00
<i>D. hansenii</i> (BFE-34)	6.76 ± 0.65 ^{fg}	26.76 ± 2.65 ^{efg}	0.95–0.96	0.00 ± 0.00 ^c	6.76 ± 0.65 ^{de}	6.76 ± 0.65 ^{cde}	26.76 ± 2.65 ^{def}	0.94–0.96
<i>D. hansenii</i> (BIOTECH 2204)	7.51 ± 1.31 ^{ef}	31.38 ± 5.80 ^{ef}	0.91–0.99	4.62 ± 5.33 ^c	6.36 ± 2.57 ^{de}	8.63 ± 3.78 ^{cde}	35.98 ± 15.62 ^{def}	0.91–0.99
<i>K. marxianus</i> (BIOTECH 2223)	5.35 ± 0.71 ^{ghi}	20.38 ± 3.75 ^{efg}	0.93–1.00	2.72 ± 0.68 ^c	4.84 ± 0.85 ^c	4.84 ± 0.85 ^{cde}	18.43 ± 3.91 ^{ef}	0.92–1.00
<i>M. guilliermondii</i> (LJY3)	8.93 ± 1.58 ^e	43.64 ± 8.21 ^{cd}	0.91–0.97	2.13 ± 3.82 ^c	8.23 ± 1.00 ^d	10.37 ± 4.23 ^{cd}	50.73 ± 21.19 ^d	0.92–0.98
<i>P. anomala</i> (BIOTECH 2205)	5.81 ± 0.47 ^{ghi}	24.81 ± 1.78 ^{efg}	0.99–1.00	0.56 ± 1.13 ^c	5.64 ± 0.47 ^{de}	6.21 ± 1.05 ^{cde}	26.51 ± 4.27 ^{def}	0.98–1.00
<i>P. fermentans</i> (BFE-38)	6.57 ± 0.75 ^{fg}	32.21 ± 3.88 ^{de}	0.97–0.99	0.75 ± 1.51 ^c	6.43 ± 1.01 ^{de}	7.19 ± 0.56 ^{cde}	35.21 ± 2.59 ^{def}	0.97–1.00
<i>P. fermentans</i> (LJY6)	3.82 ± 0.09 ⁱ	18.05 ± 0.37 ^{fg}	0.93–0.98	0.00 ± 0.00 ^c	3.82 ± 0.09 ^c	3.82 ± 0.09 ^c	18.05 ± 0.37 ^{ef}	0.91–0.97
<i>S. cerevisiae</i> (BFE-39)	4.26 ± 0.83 ^{hi}	20.96 ± 3.79 ^{efg}	0.95–0.98	0.00 ± 0.00 ^c	4.26 ± 0.83 ^c	4.26 ± 0.83 ^{de}	20.96 ± 3.78 ^{def}	0.93–0.98
<i>S. cerevisiae</i> (BIOTECH-2019)	6.85 ± 0.50 ^{fg}	29.21 ± 2.27 ^{efg}	0.97–1.00	0.00 ± 0.00 ^c	6.64 ± 0.66 ^{de}	6.64 ± 0.66 ^{cde}	28.29 ± 2.95 ^{def}	0.93–0.98
<i>T. delbrueckii</i> (BFE-37)	6.02 ± 0.70 ^{ghi}	26.23 ± 2.98 ^{efg}	0.94–0.99	0.00 ± 0.00 ^c	6.02 ± 0.70 ^{de}	6.02 ± 0.70 ^{de}	26.23 ± 2.98 ^{def}	0.93–0.98
<i>T. delbrueckii</i> (LJY5)	3.86 ± 0.77 ⁱ	17.34 ± 5.99 ^g	0.93–0.95	0.00 ± 0.00 ^c	3.86 ± 0.77 ^c	3.86 ± 0.77 ^c	17.35 ± 5.99 ^f	0.94–0.99
<i>T. cutaneum</i> (BIOTECH-2089)	26.74 ± 0.39 ^a	110.01 ± 2.36 ^b	0.94–0.99	15.05 ± 17.60 ^b	23.49 ± 4.17 ^a	38.54 ± 13.45 ^a	159.91 ± 60.43 ^b	0.98–0.99
Composite inoculum	22.76 ± 0.44 ^c	99.96 ± 3.87 ^b	0.93–0.95	0.00 ± 0.00 ^c	22.76 ± 0.46 ^a	22.76 ± 0.46 ^b	99.97 ± 16.08 ^c	0.98–0.99

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

¹ Averages of 4 values ± SD obtained from 2 independent run.

² Inactivation kinetic parameters of the yeast isolates were determined using 2 methods, namely the linear regression using Microsoft Excel and fitting into the Baranyi and Roberts (1994) model using the DMFIT MS Excel add in (Quadram Research, Bioscience, UK).

³ The decimal reduction time (D, s) is the UV-C exposure time necessary to achieve a 90% reduction in the population of each yeast isolate in orange juice.

⁴ Whenever the Baranyi and Roberts (1994) model fit yielded an inactivation lag, the corrected D value (D', s) was calculated. The D' value is equivalent to the sum of the lag and D values and is similarly defined as the UV-C exposure time necessary to achieve a 90% reduction in the population of each yeast isolate in orange juice.

⁵ In both calculation methods, the ultraviolet-c decimal reduction energy (D_{UV-C} , mJ/cm²) was determined as the product of the D or D' values and the UV-C irradiance ($3.42\text{--}4.99 \text{ mW/cm}^2$) measured on the surface of the irradiated juice. The D_{UV-C} value is defined as the UV-C energy dose necessary to achieve a 90% reduction in the population of each yeast isolate in orange juice.

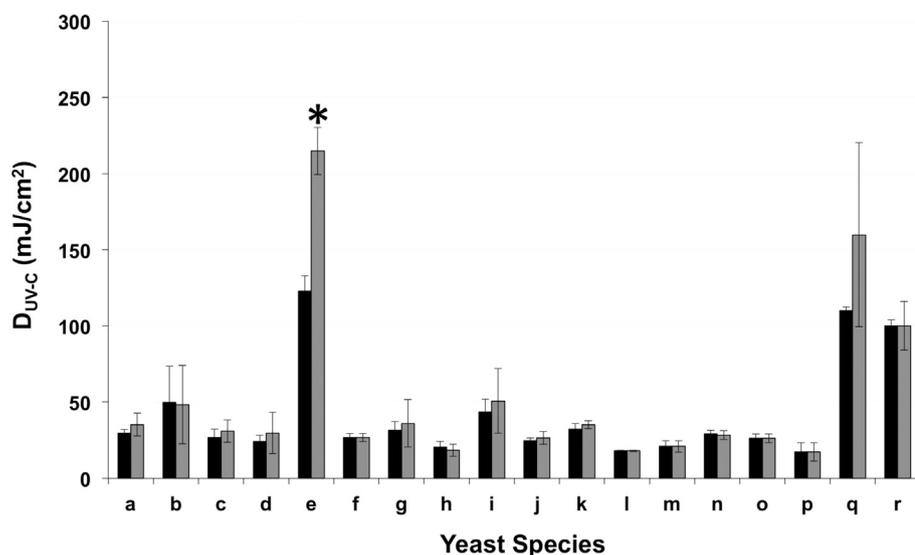


Fig. 4. Comparison of D_{UV-C} values of various yeast isolates in coconut liquid endosperm calculated using linear regression (black bars) and Baranyi and Roberts (1994) model fitting (grey bars). (a) *C. parapsilosis* (LUSC1), (b) *C. pseudointermedia* (LJY2), (c) *C. tropicalis* (BIOTECH 2085), (d) *C. lusitanae* (BFE-36), (e) *C. albidus* (LJY1), (f) *D. hansenii* (BFE-34), (g) *D. hansenii* (BIOTECH 2204), (h) *K. marxianus* (BIOTECH 2223), (i) *M. guilliermondii* (LJY3), (j) *P. anomala* (BIOTECH 2205), (k) *P. fermentans* (BFE-38), (l) *P. fermentans* (LJY3), (m) *S. cerevisiae* (BFE-39), (n) *S. cerevisiae* (BIOTECH 2019), (o) *T. delbrueckii* (BFE-37), (p) *T. delbrueckii* (LJY5), (q) *T. cutaneum* (BIOTECH 2089), and (r) composite of all 17 isolates. Asterisk denotes significant difference ($P < 0.05$) between D_{UV-C} values determined using the two methods.

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