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Ultraviolet-C resistance of selected spoilage yeasts in orange juice

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ABSTRACT

This study determined the ultraviolet-C (UV-C) dose necessary to reduce 90% population ($D_{UV\text{-}C}$) of 17 spoilage yeasts and their composited inoculum in orange juice (pH 3.71, 11.60 °Brix, 0.55% citric acid, 2.46% w/v insoluble solids). Growth parameters of all test yeasts were first established to standardize the growth stage of the cells prior to harvesting and eventual UV-C challenge studies. Approximately 4–5 log CFU/ml cells in the mid-stationary growth phase (30.3 t0 39.9 h, 25 °C) were suspended in 4 ml turbulent flowing juice and subjected to UV-C irradiation at an incident surface irradiance of 3.64–4.97 mW/cm^2. The inactivation rates of each yeast and their composited inoculum were determined using 2 methods namely, the linear regression and Baranyi and Roberts (1994) model-fitting. Results showed that the yeasts exhibited either log-linear or biphasic inactivation behavior with downward concavity or inactivation lag. Regardless of the method of determination, *Cryptococcus albidus* (LJY1) exhibited the significantly greatest ($p < 0.05$) UV-C resistance with $D_{UV\text{-}C}$ values of 1924.31 and 2174.63 mJ/cm^2. On the other hand, *Candida parapsilosis* was determined to be least resistant with a $D_{UV\text{-}C}$ values of 245.83 and 357.88 mJ/cm^2. Majority of the $D_{UV\text{-}C}$ values determined from the model-fitting were greater than those calculated from linear regression. However, only those determined for the composited inoculum were significantly different. The results of this study address knowledge gaps pertinent to the UV-C resistance of less studied spoilage yeast, and help in better understanding the utility of this non-thermal food processing technology.

1. Introduction

Fruit and vegetable juices are commonly thermally processed because the technology is simple and accessible to food processors (Rosnah et al., 2013). However, the application of high temperature to heat-sensitive raw materials is detrimental to the nutritional and sensory properties of the finished products (Choi and Nielsen, 2005; Rawson et al., 2011). Fruit juice color is one of the more commonly reported characteristics significantly affected by thermal processing in pineapple (55–95 °C for 90 min), orange (95–105 °C, 10 s) and grapefruit juices (47–97 °C, 5–60 min) (Gabriel et al., 2017a; Rattanathanalerk et al., 2005; Torres Gama and de Sylos, 2007). Ascorbic acid content has also been shown to be negatively affected by thermal processing as well (Gabriel et al., 2015a, 2015b). Such limitations of thermal processing has thus become impetus for the development nonthermal alternatives for fruit juice processing.

Ultraviolet (UV) irradiation has already been approved by the United States Food and Drug Administration (USFDA) and United States Department of Agriculture (USDA) as an alternative food processing technology for fruit juices (Federal Register, 2001). The ultraviolet region is a germicidal range of the electromagnetic spectrum at 200–800 nm (Sastry et al., 2000). It is composed of three regions, namely the long wave UV-A region (320–400 nm), the medium wave UV-B region (280–320 nm), and the short wave UV-C region (200–280 nm) (Falguera et al., 2011). The UV-C region has been reported to be able to induce more mutagenic lesions to the DNA namely the cyclobutane pyrimidine dimers (CPD), pyrimidine 6-4 pyrimidone photoproducts (6-4PPs) and its Dewar isomers (Gayán et al., 2014; Sinha and Häder, 2002). These lesions further lead to disruption in DNA transcription and replication, leading to mutations and eventual death (Sinha and Häder, 2002). The potential of UV-C processing of fruit juices has already been explored by several researchers (Gabriel, 2015; Gabriel and Colambo, 2016; Kaya and Unluturk, 2016; Oteiza et al., 2010; Santhirasegaram et al., 2015). Moreover, the UV-C processing of fruit juices promises lower financial cost compared to thermal processing and other novel processing technologies (Adzahan et al., 2011; Rodriguez-Gonzalez et al., 2015).

However, relatively fewer studies have been conducted to determine inactivation kinetic parameters of spoilage yeasts in UV-C treated juices (Fredericks et al., 2011; Gabriel, 2012; Gouma et al., 2015; Guerrero-Beltrán et al., 2009; Kaya et al., 2015). Most of the studies conducted on UV inactivation is on the characterization of the UV-C decimal reduction dose in pathogenic bacteria (Gabriel, 2012; Gayán et al., 2014; Guerrero-Beltrán, 2004; Quintero-Ramos et al., 2004), notwithstanding the fact that spoilage yeasts are more resistant to ultraviolet irradiation. Common spoilage yeast species found in citrus

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juices, coconut liquid endosperm, cashew juice, black currant, and pineapple include *Candida intermedia, C. parapsilosis, C. tropicalis, Clavispora lusitaniae, Debaryomyces hansenii, Pichia anomala, P. fermentans, Saccharomyces cerevisiae, Trichosporon mucoides, Torulaspora delbrueckii, Meyerozyma guilliermondii, Kluyveromyces marxianus, and Cryptococcus albidus*, (Arias et al., 2002; Corte et al., 2015; Maciel et al., 2013; Senses-Ergul et al., 2006). Spoilage yeasts are responsible for the alteration and loss of quality attributes of fruit juices such as aroma, flavor, color, taste, and loss or presence of cloudiness (Bevilacqua et al., 2011). Yeast spoilage of fruit juices are frequently characterized by the presence of carbon dioxide, alcohol, and fermented flavor due to organic acids produced, and occasional flocculation and pellicle formation (Aneja et al., 2014). Gayán, Serrano, Pagan, Alvarez, and Condon (2015) explained that in order for the UV-C technology to be accepted by and effectively transferred to the industry, the need to further understand UV-C resistance and inactivation behavior of microorganisms of significance to food quality and safety must first be addressed. Thus this study was conducted to determine and compare the UV-C resistance of selected spoilage yeasts in an orange juice suspending medium. The UV-C decimal reduction dose or $D_{UV\text{-}C}$ values necessary to inactivate 1 log (90%) of the population were determined, the results of which may help in further understanding the utility of this non-thermal technology for food processing.

2. Materials and methods

2.1. Fruit juice suspending medium

A commercially available orange juice (Harvey Fresh 100% orange juice, Parmalat Australia Pty. Ltd., Australia) was used for the inactivation studies as suspending medium. The physicochemical properties such as pH, titratable acidity, UV-C absorption coefficient, soluble solids (TSS, °Brix) and insoluble solids were determined. The total soluble solids (TSS) and pH were measured using handheld refractometer (Atago, Tokyo, Japan) and pH meter (Eutech pH 700, Eutech Pte., Ltd., Singapore), respectively. Titratable acidity (TA, % citric acid) was determined following the official method described by AOAC Method 942.15 (AOAC, 2000) using colorimetric titration method with 0.1 N NaOH (RCI Labscan, Thailand) until phenolphthalein endpoint, indicated by a faint pink color which persisted for 30 s. The suspended insoluble solids was determined by passing 1 mL of orange juice through 0.47 μm pore-sized filter (Advantech, Toyo Roshi Kaisha, Ltd. Japan). The filter paper with the residue was dried for 8–12 h in a desiccator until constant weight was achieved for three consecutive measurements. The dried residue represented the insoluble solids fraction of the juice and the result was expressed as % weight by volume (%w/v). The UV absorption coefficient (ε) was determined by measuring the absorbance of diluted juice samples at 254 nm and 1 cm path length using a UV/Vis Double Beam spectrophotometer (Unico SQ-4802, United Products & Instruments, Inc. New Jersey, USA). The ε was determined from the slope of initial linear part of the absorbance vs. juice concentration (Fig. 1a). Prior to being used in the experiments, the orange juices were analyzed for background microflora. The aerobic bacteria and yeast and mold counts were below detection limit (< 1.0 log CFU/ml).

2.2. Test spoilage yeast and culture maintenance

The study tested 17 strains of spoilage yeasts (Table 1) isolated from a variety of spoiled fruit and other processed food products. These included three isolates of *Candida* spp. and *Pichia* spp., two isolates of *Debaryomyces hansenii,* and *Saccharomyces cerevisiae*, and *Torulaspora delbrueckii*. The tested yeasts also included a strain each of *Clavispora lusitanae, Cryptococcus albidus, Kluyveromyces marxianus, Meyerozyma guillermondi,* and *Trichosporon cutaneum*. Prior to being used in the UV-C inactivation studies, each of the test strains were subjected to a series of activation, enrichment, and working culture preparation. Activation from freeze-dried, frozen or refrigerated stock cultures was done by inoculating cells to a laboratory-compounded Yeast Extract Peptone Glucose Broth (YPGB) composed of 0.5% yeast extract (HiMedia Laboratories Pvt. Ltd., Mubai, India), 0.1% peptone (HiMedia Laboratories Pvt. Ltd., Mubai, India), and 2.0% glucose (Sigma-Aldrich, Missouri, USA). The inoculated YPGB tubes were then incubated at 25 °C for 24–48 h. Each of the activated yeast cultures was enriched by transferring a loopful of cells into YPGB, which was thereafter subjected to incubation at 25 °C for 24–48 h. Working cultures of each of the yeast strains were prepared by streaking cells onto Potato Dextrose Agar slant (PDA, HiMedia Laboratories Pvt. Ltd., Mubai, India) which were first incubated at 25 °C for 24–48 h prior to storage at 4 °C until further used in the study. Fresh working cultures were made ever 14 d following the previously described activation and enrichment protocols.

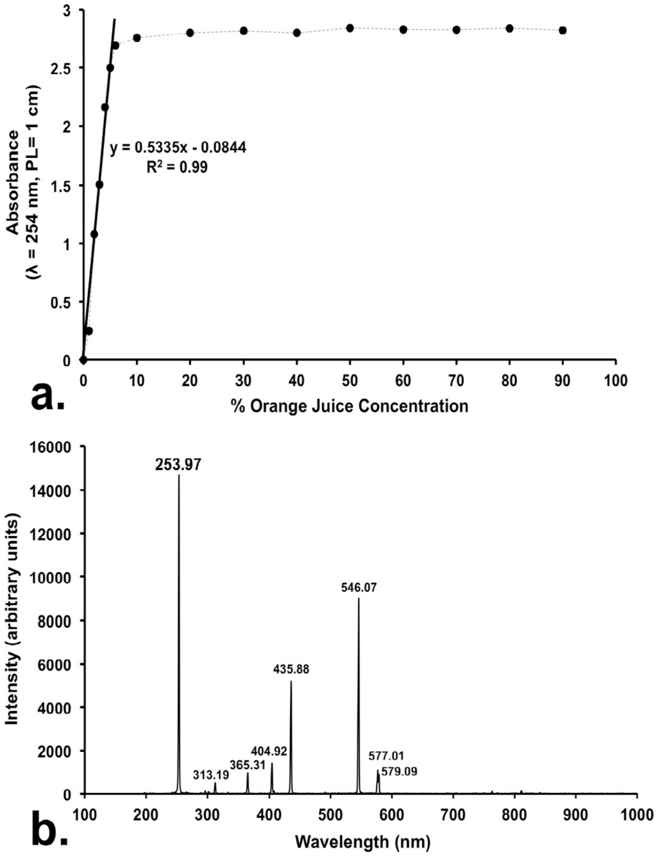

Fig. 1. Optical characteristics of the test orange juice and UV-C lamp. (**a**) A plot of orange juice concentration vs. absorbance for the determination of the percent solution extinction coefficient ($\varepsilon_{percent}$, slope of the regressed line) from which the extinction coefficient was determined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

2.3. Growth curve and growth kinetic parameter determination of spoilage yeasts

In order to minimize the effect of the growth phase of cells on the UV-C resistance of the yeasts, the growth curves and growth kinetic parameters of the isolates were determined. Specifically, the mid-stationary phase of growth was determined for each of the yeast strains. A loopful of inoculum was obtained from each of the refrigerated working cultures and subjected to the previously described activation and enrichment protocols. For the growth curve establishment, 0.4 mL aliquot was obtained from an enrich culture and was transferred into 49.6 mL sterile YPGB. The YPGB culture was then incubated at 25 °C for 48 h.

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

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

During the incubation time, an aliquot of 1 mL was obtained at 0, 1, 2, 4, 8, 12, 24, 30, 36, and 48 h respectively. The withdrawn sample was then subjected to serial 10-fold dilutions with sterile peptone water (PW, HiMedia Laboratories Pvt. Ltd., Mubai, India) and surface-plated onto pre-solidified PDA acidified with 10% tartaric acid (RTC Laboratory Services Supply House, Quezon City, Philippines). Populations were determined and expressed as log CFU/mL after incubation at 25 °C for 48 h. The growth curves of the yeasts were plotted using the Dynamic Modelling Fit Version 3.0 (DMFit 3.0, Quadram Reasearch Bioscience UK), which is based on the work of Baranyi and Roberts (1994). The growth kinetic parameters including lag time (t_{lag}), growth rate (k_G), and final population (Pop_{fin}) at stationary phase, were determined using the same freeware. Moreover, the time required to mid stationary phase (MSP) 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).

2.4. Orange juice inoculation and UV-C inactivation studies

Prior to UV-C inactivation studies, yeasts cells were activated in YPGB at 25 °C for 48 h and thereafter enriched in YPGB at 25 °C until the predetermined MSP. Cells were then harvested by spinning 1.0 mL aliquot of the enriched suspension on a bench top centrifuge (Cole Palmer, Illinois, USA) at 8000 rpm for 10 min. The supernatant liquid was then decanted while the pelleted cell was resuspended in 1.0 mL orange juice. The resuspended cells were allowed to acclimatize in orange juice for 5 min prior to inactivation studies.

A 0.04 mL aliquot of acclimatized cells in orange juice was diluted with 3.96 mL uninoculated orange juice and transferred into a 35-mm sterile plastic Petri dish. This was done to introduce an initial inoculum population of approximately 4–5 log CFU/mL. A 1.25-cm magnetic spin bar was placed at the center of the dish to introduce turbulent flow during UV-C exposure. After placing the spin bar, an average liquid sample thickness of 7 mm was recorded. The inoculated juice was then subjected to UV-C irradiation by placing each plate on a magnetic stir plate under a fabricated UV-C box with three 15-W mercury vapor lamps as UV-C light source (Sankyo Denki, Japan) at a lamp-to-sample surface distance of 100 mm. A sample of the UV-C lamp used in this study was subjected to optical emission spectroscopy that confirmed the 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 1.09 nm in the range of 200–1100 nm (Fig. 1b).

During the UV-C exposure, the inoculated juice was stirred at maximum rotational speed of 1500 rpm to introduce turbulent flow and allow even exposure to UV radiation. Simultaneous radiometric readings (UVX Radiometer, UVP, Upland, California) showed that the juice surface received an average UV-C incident surface irradiance of 4.45 mW/cm^2. That is, 1 s of exposure results in the treatment of the juice surface with a UV-C dose of 4.45 mJ/cm^2, and longer exposure to the fixed incident surface irradiance results in greater UV-C dose to inactivate the inoculate microorganisms.

Off axis photons were not considered in the determination of the UV-C irradiance on the surface of the treated orange juice. However, in the measurement of the incident surface irradiance, the detector of the radiometer was placed in the same distance and orientation (with respect to the UV-C source) as the treated orange juice. Therefore, the study assumed that UV-C photons delivered to the surface of the orange juice were detected and measured by the radiometer. At predetermined time intervals between 0 and 1300 s, survivor enumerations were determined in the UV-C-treated juice by subjecting the sample to serial 10-fold dilutions with sterile peptone water (HiMedia Laboratories Pvt. Ltd., Mubai, India) and surface-plating onto pre-solidified and acidified PDA. Colonies were enumerated after incubation at 25 °C for 48 h. The enumerated populations were expressed as log CFU/mL. UV-C inactivation studies of all 17 tested yeasts were conducted in two independent external runs each of which had two internal replicates.

The UV-C resistance of a composite of all yeast strains was also determined in this study. The composite inoculum was prepared by first subjecting each test strain to the previously described activation and culture enrichment processes. 1 mL aliquot was then obtained from each of the enriched cultures and combined by vortex mixing in a sterile flask. 1 ml aliquot was obtained from the mixture of yeast strains and subjected to the previously described cell harvesting, acclimatization, and UV-C exposure protocols.

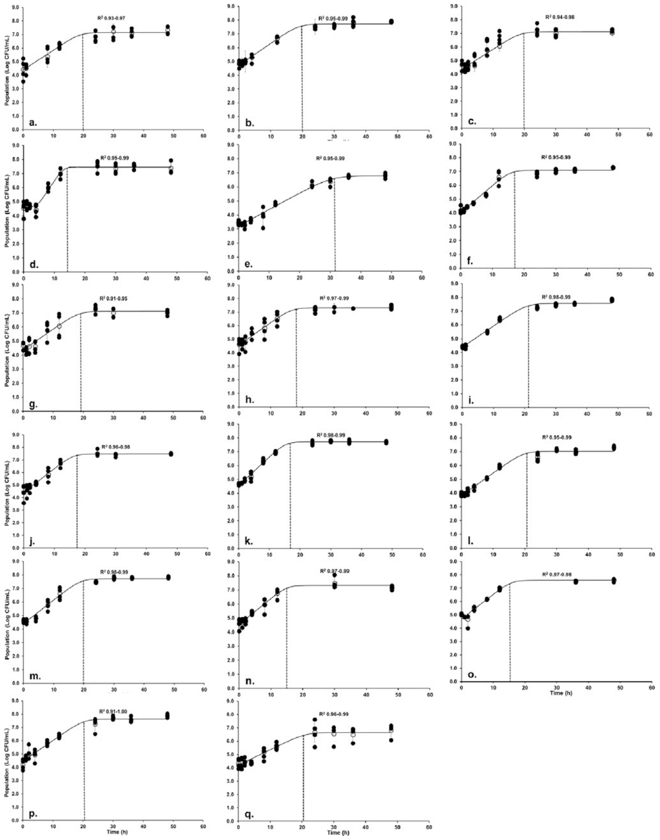

Fig. 2. Growth curves of test yeast isolates in YPG broth at 25 °C. (**a**) *C. parapsilosis* (LUSC1), (**b**) *C. pseudointermedia* (LJY2), (**c**) *C. tropicalis* (BIOTECH, 2085), (**d**) *C. lusitaniae* (BFE-36), (**e**) *C. albidus* (LJY1), (**f**) *D. hansenii* (BFE-34), (**g**) *D. hansenii* (BIOTECH 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. delbreuckii* (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.

2.5. Inactivation behaviors and inactivation kinetic parameters

In this study, the UV-C inactivation behavior and inactivation kinetic parameters of each of the test yeasts were determined using two methods, namely through linear regression using Microsoft Excel, and through Baranyi and Roberts (1994) model fitting using the DMFit 3.0 freeware package. In the linear regression method, the negative inverse of the slope of the best-fit line was determined as the decimal reduction time (D value), or the UV-C exposure time necessary to reduce the population of a test yeast strain by 1 log (90%) at a fixed incident surface irradiance. The decimal reduction dose or the UV-C dose necessary to reduce the population by 1 log ($D_{UV\text{-}C}$ value, mJ/cm^2) was also determined by multiplying the D value (s) by the average incident surface irradiance (mW/cm^2).

In the model-fitting method, the freeware was able to characterize nonlinear inactivation behavior and its inactivation kinetic parameters including inactivation lag time (t_{lag}) and inactivation rate (k_D). For linear inactivation curves, the D values were similarly determined as the negative inverse of the slope of the curve ($k_D{}^{-1}$) and the $D_{UV\text{-}C}$ values were also determined by multiplying the D values with the average UV-C incident surface irradiance. For nonlinear inactivation curve with a t_{lag}, the D' value was determined as the sum of the t_{lag} and $k_D{}^{-1}$. The $D_{UV\text{-}C}$ value was similarly determined using the previously described method.

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). The Duncan's Multiple Range Test was used for post-hoc determinations of significant differences at 95% level of significance.

3. Results and discussion

3.1. Orange juice physicochemical properties

The physicochemical properties of the suspending medium determined included pH, titratable acidity, soluble and insoluble solids, and UV-C absorption coefficient. The observed pH of 3.74 is within the pH range reported for citrus varieties such as Valencia and California, and Florida (3.69–4.34) (FDA/Center for Food Safety and Applied Nutrition, 2008; Ferrario et al., 2013; Ochoa-Velasco and Guerrero-Beltrán, 2012). Furthermore, the observed pH and soluble solids (11.73 °Bx) of the test suspending medium were also comparable with those reported for other fruit juices including commercially available native lemon, orange, mango, guava, apple, pineapple and grape juices (Gabriel, 2008). The titratable acidity of 0.55% citric acid was within the ranges reported by Gabriel et al. (2015) for freshly squeezed and processed citrus juices. The amount of insoluble solids suspended in the juice was determined to be at 2.46% w/v.

The orange juice had a UV-C absorbance of 2.85 and an equivalent UV-C transmittance of 0.14%. The ε value measured from the slope of the linear portion of the UV-C absorbance vs. concentration plot (Fig. 1a) was determined at 53.35 cm^{-1}. The UV-C absorbance characteristics of the suspending medium were determined without correcting for UV-C scattering components as similarly done in several previously reported works (Koutchma et al., 2004, 2006; 2007; Murakami et al., 2006; Oteiza et al., 2010). The ε value determined for orange juice is comparable to previously reported absorption coefficients for juices from oranges (52.4 and 71.72 cm^{-1}), *lillikoi* (11.0 cm^{-1}), and pineapple (78.0 cm^{-1}) (Castillo et al., 2006; Koutchma et al., 2007; Pataro et al., 2011). Variations in the determined and previously reported physicochemical properties may be attributed to differences in composition of the food matrices. Definition of these properties is important when establishing the efficacy of UV-C against specific organisms, as these may influence the resistance of the organism against the inactivating agent (Gayán et al., 2014; Turtoi and Borda, 2013).

3.2. Growth kinetic parameters of yeast isolates

Previous studies showed that growth phase influences the resistance of microorganisms towards different inactivation factors (Child et al., 2002; Nair and Finkel, 2004). Hence in this study, the growth kinetics of the tested yeast isolates were established to achieve uniformity in the growth stage of microorganisms prior to UV-C inactivation studies. The growth curves of the yeast isolates established in YPGB are summarized in Fig. 2, while the growth kinetic parameters are summarized in Table 2. Results showed that only 3 yeast strains exhibited growth lag ranging from 1.69 (*S. cerevisiae* BFE-39) to 3.44 h (*C. lusitaniae* BFE-36), while the other 14 strains immediately grew in YPGB. Gabriel (2012) observed the occurrence of growth lag when *C. lusitaniae, D. hansenii, T. delbrueckii, P. fermentans,* and *S. cerevisiae* when grown 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

Table 2
Growth kinetic parameters[a] 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)[b]	R^2
C. parapsilosis (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
C. pseudointermedia (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
C. tropicalis (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
C. lusitaniae (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
C. albidus (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
D. hansenii (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
D. hansenii (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
K. marxianus (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
M. guilliermondii (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
P. anomala (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
P. fermentans (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
P. fermentans (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
S. cerevisiae (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
S. cerevisiae (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
T. delbrueckii (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
T. delbrueckii (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
T. cutaneum (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 ($P > 0.05$).

[a] 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 freeware (Institute of Food Reseach, Norwich, UK).

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

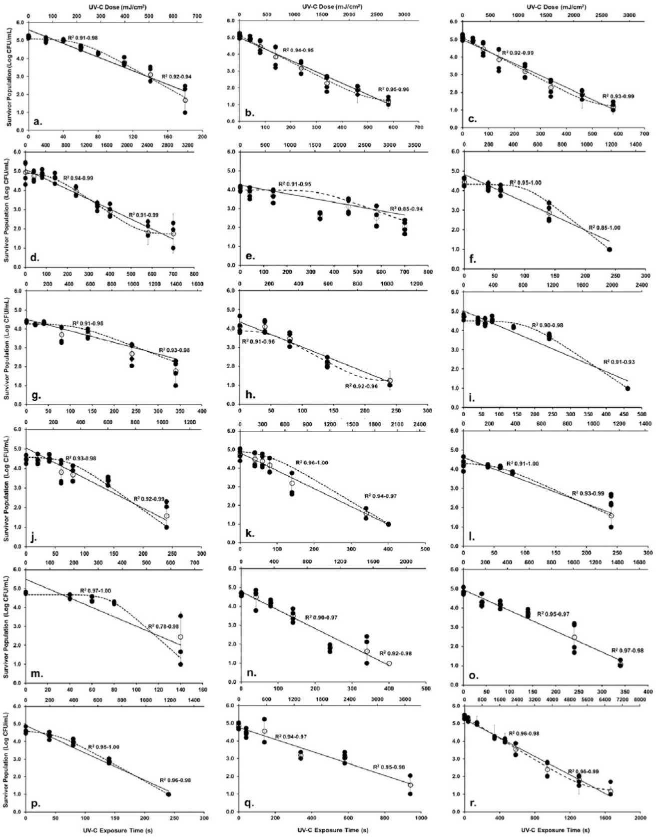

Fig. 3. UV-C inactivation curves of test yeast species in orange juice. (**a**) *C. parapsilosis* (LUSC1), (**b**) *C. pseudointermedia* (LJY2), (**c**) *C. tropicalis* (BIOTECH, 2085), (**d**) *C. lusitaniae* (BFE-36), (**e**) *C. albidus* (LJY1), (**f**) *D. hansenii* (BFE-34), (**g**) *D. hansenii* (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. delbreuckii* (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. Solid curves represent linear regression model fit. Dashed curves represent fit in the Baranyi and Roberts (1994) model.

physiological functions that are necessary for survival in the growth medium. These physiological functions precede the initiation of cellular multiplication.

It should be noted that despite having the longest growth lag time, *C. lusitaniae* (BFE-36) exhibited the significantly fastest ($p < 0.05$) growth rate of 0.61 log CFU/h. This growth rate was almost 6-fold greater than the slowest growing strain *T. cutaneum* (BIOTECH-2089) with a growth rate of 0.11 log CFU/h. The remaining 16 strains had growth rates (0.11–0.24 log CFU/h) not significantly different ($p > 0.05$) from each other. Evident in Fig. 2, majority of the yeast isolates reached the start of the stationary phase of growth after 18–20 h. The isolate observed to reach the stationary phase fastest was *C. lusitaniae* (< 15 h), while *C. albidus* was the slowest to reach stationary phase (30 h).

The stationary phase of the selected spoilage yeasts occurred earlier than some of previously reported kinetic parameters of yeasts (Herman, 2002; Uppuluri and Chaffin, 2007; Werner-Washburne et al., 1996, 1993). However, when compared with those reported by Gabriel (2012) for selected yeasts, the results of the current study are similar. Aside from interisolate and interspecies variation in the growth kinetic parameters of microorganisms, Herman (2002) and Jay et al. (2005) explained that the differences in the onset of stationary phase can be linked to the difference in the available nutrients present in the growth medium. Higher nutrient concentration in the growth medium will result in the continuous increase in cell for longer periods of time. The onset of stationary phase is an indication of the exhaustion of available nutrients resulting in the lowering of the growth rate to its minimum. In the stationary phase, physiological changes in yeasts take place, including the accumulation of glycogen, increased resistance to a variety of environmental stresses like heat shock, thickening of the cell wall, and the increased ability to survive extended periods of starvation. For each yeast isolate, the midpoint between the start of the stationary phase and the end of the last population monitoring (48 h) was the determined as the mid stationary phase MSP, which ranged from 31.6 to 39.9 h, are summarized in Table 2. Thus in the subsequent inactivation studies, each of the test yeast isolates were propagated in NBG until the predetermined MSP prior to cell harvest, orange juice inoculation, and UV-C treatment.

3.3. UV-C inactivation behaviors of spoilage yeasts in orange juice

The inactivation kinetic parameters of all test yeasts in UV-C

Table 3
Inactivation kinetic parameters[a] of spoilage yeasts in orange juice.

Yeast Isolates	Inactivation Kinetic Parameters Calculated per Method[b]							
	Linear Regression			Baranyi and Roberts (1994) Model Fitting				
	D (s)[c]	$D_{UV\text{-}C}$ (mJ/cm^2)	R^2 range	Lag (s)	D (s)	D' (s)[d]	$D_{UV\text{-}C}$[a] (mJ/cm^2)[e]	R^2 range
C. parapsilosis (LUSC1)	55.31 ± 13.71^{h}	245.83 ± 72.17^{g}	0.92–0.94	34.10 ± 39.93^{b}	47.76 ± 22.46^{e}	81.86 ± 17.05^{g}	357.88 ± 57.72^{g}	0.91–0.98
C. pseudointermedia (LJY2)	145.24 ± 7.44^{e}	677.65 ± 43.90^{d}	0.95–0.96	0.00 ± 0.00^{b}	141.24 ± 4.02^{bc}	141.24 ± 4.02^{efg}	659.15 ± 36.38^{defg}	0.94–0.95
C. tropicalis (BIOTECH, 2085)	195.47 ± 86.80^{d}	733.22 ± 290.97^{d}	0.93–0.99	0.00 ± 0.00^{b}	195.27 ± 86.56^{b}	195.27 ± 86.56^{de}	732.45 ± 290.09^{cde}	0.92–0.99
C. lusitaniae (BFE-36)	196.68 ± 52.53^{d}	801.36 ± 140.26^{d}	0.91–0.99	103.90 ± 120.01^{ab}	124.53 ± 33.49^{cd}	228.43 ± 87.78^{cd}	922.27 ± 271.93^{cd}	0.94–0.99
C. albidus (LJY1)	409.42 ± 54.07^{a}	1924.31 ± 198.88^{a}	0.85–0.94	164.73 ± 180.70^{a}	299.69 ± 88.46^{a}	464.42 ± 107.81^{a}	2174.63 ± 422.30^{a}	0.91–0.95
D. hansenii (BFE-34)	67.74 ± 2.20^{h}	334.25 ± 10.20^{efg}	0.89–1.00	25.52 ± 51.04^{b}	60.62 ± 12.82^{de}	86.14 ± 38.31^{g}	424.65 ± 187.02^{efg}	0.95–1.00
D. hansenii (BIOTECH 2204)	120.58 ± 13.20^{efg}	492.22 ± 33.68^{e}	0.93–0.98	83.47 ± 96.40^{ab}	93.92 ± 21.50^{cde}	177.39 ± 76.58^{def}	708.70 ± 254.53^{cdef}	0.91–0.98
K. marxianus (BIOTECH 2223)	68.94 ± 19.50^{h}	293.29 ± 84.70^{fg}	0.91–0.96	18.64 ± 37.29^{b}	62.77 ± 25.51^{de}	81.42 ± 27.04^{g}	345.96 ± 114.37^{g}	0.92–0.96
M. guilliermondii (LJY3)	136.01 ± 8.74^{ef}	652.73 ± 40.19^{d}	0.91–0.93	89.39 ± 103.29^{ab}	109.95 ± 38.62^{cde}	199.35 ± 65.08^{de}	956.89 ± 313.12^{c}	0.90–0.98
P. anomala (BIOTECH 2205)	83.52 ± 21.74^{gh}	350.53 ± 78.68^{efg}	0.93–0.98	36.28 ± 39.92^{b}	74.68 ± 31.67^{de}	110.96 ± 10.61^{fg}	469.92 ± 60.56^{efg}	0.92–0.99
P. fermentans (BFE-38)	87.15 ± 12.45^{fgh}	431.89 ± 62.20^{ef}	0.94–0.97	60.73 ± 70.13^{ab}	68.97 ± 33.44^{de}	129.70 ± 36.90^{efg}	642.51 ± 182.08^{defg}	0.96–1.00
P. fermentans (LJY6)	87.41 ± 26.71^{fgh}	409.29 ± 106.18^{efg}	0.93–0.99	19.40 ± 24.18^{b}	83.24 ± 31.06^{cde}	102.65 ± 13.01^{fg}	484.60 ± 51.01^{efg}	0.91–1.00
S. cerevisiae (BFE-39)	64.61 ± 23.44^{h}	295.71 ± 107.57^{fg}	0.78–0.98	37.80 ± 43.73^{b}	51.52 ± 37.35^{e}	89.33 ± 6.40^{g}	408.65 ± 28.70^{fg}	0.97–1.00
S. cerevisiae (BIOTECH-2019)	96.55 ± 14.95^{fgh}	430.43 ± 69.34^{ef}	0.92–0.98	0.00 ± 0.00^{b}	96.62 ± 15.04^{cde}	96.62 ± 15.04^{g}	430.75 ± 69.77^{efg}	0.90–0.97
T. delbrueckii (BFE-37)	90.61 ± 3.72^{fgh}	380.47 ± 33.78^{efg}	0.97–0.98	0.00 ± 0.00^{b}	90.73 ± 3.92^{cde}	90.73 ± 3.92^{g}	381.00 ± 34.61^{g}	0.95–0.97
T. delbrueckii (LJY5)	64.64 ± 4.44^{h}	319.89 ± 20.56^{efg}	0.96–0.98	25.88 ± 30.83^{b}	58.86 ± 8.37^{e}	84.74 ± 23.82^{g}	419.34 ± 117.19^{fg}	0.95–1.00
T. cutaneum (BIOTECH-2089)	297.66 ± 23.32^{c}	1247.19 ± 47.86^{c}	0.95–0.98	0.00 ± 0.00^{b}	295.97 ± 23.74^{a}	295.97 ± 23.74^{bc}	1240.03 ± 49.53^{b}	0.94–0.97
Composite inoculum	351.67 ± 8.71^{b}	1740.84 ± 46.30^{b}	0.96–0.98	0.00 ± 0.00^{b}	349.90 ± 42.14^{a}	349.90 ± 42.14^{b}	1398.74 ± 143.87^{b}	0.96–0.99

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

[a] Averages of 4 values ± SD obtained from 2 independent run.

[b] 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 freeware (Institute of Food Reseach, Norwich, UK).

[c] 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.

[d] 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.

[e] In both calculation methods, the ultraviolet-c decimal reduction energy ($D_{UV\text{-}C}$, mJ/cm^2) was determined as the product of the D or D′ values and the UV-C irradiance (3.64–4.97 mW/cm^2) measured on the surface of the irradiated juice. The $D_{UV\text{-}C}$ 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.

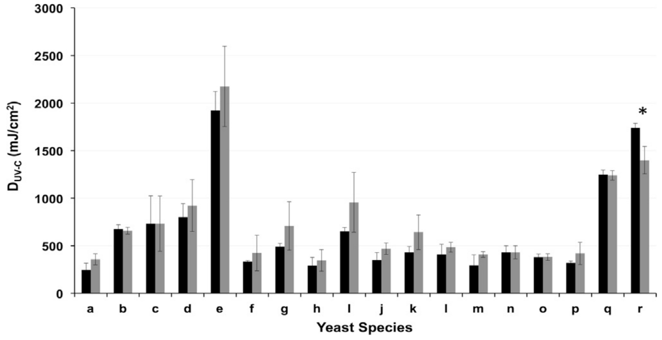

Fig. 4. Comparison of $D_{UV\text{-}C}$ values of various yeast isolates in orange juice calculated using linear regression (black bars) and Baranyi and Roberts (1994) model fitting (grey bars). (**a**) *C. parapsilosis* (LUSC1), (**b**) *C. pseudointermedia* (LJY2), (**c**) *C. tropicalis* (BIOTECH, 2085), (**d**) *C. lusitaniae* (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. delbreuckii* (LJY5), (**q**) *T. cutaneum* (BIOTECH, 2089), and (**r**) composite of all 17 isolates. Asterisk denotes significant difference ($p < 0.05$) between $D_{UV\text{-}C}$ values determined using the two methods.

irradiated orange juice were determined through linear regression and Baranyi and Roberts (1994) model-fitting. The inactivation curves that resulted from these two methods are summarized in Fig. 3. The inactivation kinetic parameters are presented in Table 3. Linear regression fitting showed that 15 out of 18 of the tested yeast strains, including the composited strains, exhibited a logarithmic-linear UV-C inactivation behavior with inactivation curves having $R^2 > 0.90$. In some inactivation runs, *S. cerevisiae* (BFE-39), *C. albidus* (LJY1), and *D. hansenii* (BFE-34) had inactivation curves with $R^2 < 0.90$. Despite this observation, fitting the survivor populations to the Baranyi and Roberts (1994) model showed that 12 yeast strains exhibited a biphasic inactivation behavior composed of an initial inactivation lag where minimal cells are inactivated, followed by a fast logarithmic-linear inactivation phase. The significantly longest mean inactivation lag was determined for *C. albidus* (LJY1) at 164.73 s, while the shortest inactivation lag was observed in *K. marxianus* (BIOTECH-2223) at 18.64 s. The inactivation data of all tested yeast strains, including the composited inoculum, fitted well in the Baranyi and Roberts (1994) model with R^2 values ranging from 0.90 to 1.00.

Similar to what has been observed in this current work, the UV-C inactivation behavior of spoilage yeasts in fruit juices vary in previously reported studies. Gabriel (2012) described a biphasic UV-C inactivation of *T. delbrueckii* and linear inactivation behavior of *D. hansenii* and *P. fermentans* in apple juice. Inactivation lags were also observed UV-C-treated *S. cerevisiae, Saccharomyces bayanus, Zygosaccharomyces bailii, Dekkera bruxellensis,* and *Dekkera anomala* in McIlvane buffer (Gouma et al., 2015). The inactivation lag times observed for *C. albidus* and *M. guilliermondii* were longer than the lag times reported by Gouma et al. (2015). The occurrence of a shoulder or an inactivation curve with downward concavity demonstrates that it takes a progressively shorter time to inactivate the same fraction of the population, possibly through the accumulation of damages that lowers the resistance of the cells (Peleg, 2006; Peleg and Cole, 1998). The occurrence of this biphasic inactivation behavior characterized by the initial inactivation lag was also previously explained to be due to presence of clumps of cells, and re-synthesis of critical cellular components necessary for survival (Bevilacqua et al., 2015; Geeraerd et al., 2000; Xiong et al., 1999). Nonlinear inactivation behaviors have also been attributed to the heterogeneity of cellular susceptibility towards the inactivating agent and cellular aggregation (McKellar and Lu, 2004; Yousef and Marth, 1998).

3.4. $D_{UV\text{-}C}$ dose of spoilage yeasts in orange juice

The inactivation kinetic parameters of all test yeast strains and their composite, obtained through linear regression and the Baranyi and Roberts (1994) model fitting are presented in Table 3. The $D_{UV\text{-}C}$ values were determined from the product of the D values and the measured UV-C incident surface irradiance. This study did not conduct chemical and biodosimetry to confirm the exact amount of UV-C dose received by the cells suspended beneath the surface film of the treated juice. The UV-C incident surface irradiance was reported as measured by the radiometer. In this study, it was assumed that UV-C inactivation beneath the surface of the orange juice was negligible. That is, microbial inactivation observed in this study was assumed to have taken place on the surface, which was enhanced by the small treated volume (4 ml) and the introduction of turbulent flow during treatment.

In the linear regression method, the calculated D ranged from 55.31 (*C. parapsilosis* (LUSC1)) to 409.42 s (*C. albidus* (LJY1)). The equivalent $D_{UV\text{-}C}$ values ranged from 245.83 to 1924.31 mJ/cm^2. The composited yeast strains exhibited UV-C resistance next to the most resistant *C. albidus*. More than half of the challenge yeasts including *D. hansenii* (BIOTECH 2204), *P. fermentans* (BFE-38), *S. cerevisiae* (BIOTECH, 2019), *P. fermentans* (LJY6), *T. delbruecki* (BFE-37), *P. anomala* (BIOTECH 2205), *D. hansenii* (BFE-34), *T. delbruecki* (LJY5), *S. cerevisiae* (BFE-39), and *K. marxianus* (BIOTECH 2223) had comparable UV-C resistance. Among the tested strains from the same genus, only *Candida* species exhibited significant interspecies variation in UV-C resistance, with *C. tropicalis* (BIOTECH, 2085) exhibiting > 66% more resistance than *C. parapsilosis* (LUSC1).

A similar trend in UV-C resistance was observed when the survivor populations over UV-C treatment data were fitted in the Baranyi and Roberts (1994) model. *C. albidus* was found most significantly resistant with a $D_{UV\text{-}C}$ of 2174.63 mJ/cm^2. This was followed by the composited inoculum with a $D_{UV\text{-}C}$ of 1398.74 mJ/cm^2. *C. parapsilosis* was similarly found least resistant towards UV-C, which was 6-fold less resistant than *C. albidus*. Similarly, more than half of the rest of the yeast strains had less varying a $D_{UV\text{-}C}$. When the UV-C resistance parameters calculated using the two methods were compared (Fig. 4), most of the $D_{UV\text{-}C}$ values determined from the Baranyi and Roberts (1994) model-fitting were greater than those determined using linear regression. Furthermore, only the $D_{UV\text{-}C}$ values determined using the 2 methods for the composited yeast trains were significantly different.

The UV-C inactivation energies of the spoilage yeasts established in this study were higher than those of pathogenic and spoilage bacteria in apple juice (0.04 mJ/cm^2), white grape juice (0.06–0.14 mJ/cm^2), coconut liquid endosperm (3.2–3.5 mJ/cm^2, 7.63–14.56 mJ/cm^2) and liquid egg white (170.71–240.33 mJ/cm^2) (Baysal et al., 2013; Gabriel et al., 2017b; Gabriel and Colambo, 2016). In addition, the inactivation energies determined in this study were higher against stressed *Salmonella enterica*, some of which exhibited heterologous adaptation towards UV-C (Estilo and Gabriel, 2017; Gabriel et al., 2016). Moreover, the inactivation energies of most of the tested yeasts were higher compared to the inactivation *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).

Spoilage yeasts are known to be more resistant to ultraviolet-C radiation than pathogenic bacteria, but not as resistant as other microorganisms such as algae, bacterial spores and molds (Guerrero-Beltrán, 2004). The greater $D_{UV\text{-}C}$ of yeasts compared to bacteria reflect the dose necessary to penetrate the eukaryotic yeast cells to disrupt essential DNA biochemistry, and eventually induce mutagenic lesions that result in inactivation (Bolton, 2004; Sinha and Häder, 2002). 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 *C. parapsilosis* isolate when suspended in *calamansi* juice drink ($\varepsilon = 4.83\ \mathrm{cm}^{-1}$) with a $D_{UV\text{-}C}$ of 183.85 $\mathrm{mJ/cm}^2$ (Gabriel et al., 2018).

S. cerevisiae are commonly used as reference organism, and as such, comparison of the inactivation behavior and kinetics with other yeast species is limited. To the investigators' knowledge, this is the first time that a report on the UV-C inactivation behaviors and energies of *K. marxianus, T. cutaneum, P. anomala, C. albidus,* and *M. guilliermondii* in orange juice is being made. Moreover, the results provide viable reference organisms for establishing ultraviolet-C process in fruit juices and other food products highly susceptible to spoilage. Gabriel et al. (2018) established an UV-C pasteurization process for *calamansi* (Philippine lemon) juice drink based on the inactivation kinetics of *C. parapsilosis* isolated from a spoiled beverage sample. With the results established in this current study, the previously established process schedule may still be further validated and improved since the *C. parapsilosis* was found to be significantly less resistant to UV-C than other spoilage strains.

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