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ORIGINAL ARTICLE

The impact of UV-C irradiation on spoilage microorganisms and colour of orange juice

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Abstract The effect of UV-C irradiation on inactivation of spoilage microorganisms and colour of freshly squeezed orange juice were investigated. Orange juice samples were intentionally fermented in order to increase the natural microflora which were mostly composed of yeasts and then exposed to UV-C irradiation at an intensity level of 1.32 mW/cm^2 and sample depth of 0.153 cm for several exposure times by using a collimated beam apparatus. Applied UV dose was in the range of 0 and 108.42 mJ/cm^2. Resistance of yeast to UV light and existence of suspended particles limited the effectiveness of the process. Survival data obtained for yeasts was either described by the Weibull or traditional first-order model and goodness-of-fit of these models was investigated. Weibull model produced a better fit to the data with higher adjusted determination coefficient (R^2_{adj}) and lower mean square error (MSE) values which were 0.99 and 0.003, respectively. Time and UV dose of first decimal reduction were obtained as 5.7 min and 31 mJ/cm^2, respectively. The data suggests that biodosimetric studies performed by using inoculated microorganisms for assesment of the efficiency of UV irradiation treatment in the shelf life extension of juices must be carefully evaluated. UV-C irradiation had no influence on the colour of orange juice.

Keywords UV-C irradiation · Orange juice · Spoilage yeast · Inactivation · Colour

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Introduction

Consumption of fresh fruit juices has been increasing since plenty of different enzymes, vitamins, polyphenolic compounds and other nutrients are present in unprocessed juices (Jo and Lee 2011). Conventional heat pasteurization is the technique applied to reduce the number of pathogens such as *Escherichia coli* O157:H7, *Salmonella* sp., *Listeria monocytogenes* and *Cryptosporidium parvum* in various types of juices (Tandon et al. 2003; Silva and Gibbs 2004). The adverse effects of thermal pasteurization on food quality and increasing trend towards more healthy and safer products arouse the need to new technologies in food processing i.e. high pressure treatment, pulsed electric field treatment etc. (Bates et al. 2001; Pala and Toklucu 2011). UV-C irradiation is a non-thermal and low cost operation (Tahiri et al. 2006; Basaran-Akgul et al. 2009). As a non-thermal preservation method, UV-C light irradiation is of interest to the food industry. UV light is one portion of the electromagnetic spectrum. The wavelength for UV processing ranges between 100 and 400 nm. This range can be subdivided into four groups which are UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm) and the vacuum UV range (100–200 nm). UV-A is responsible for tanning whereas UV-B causes skin burning and eventually leads to skin cancer. On the other hand UV-C is called as the germicidal range and the vacuum UV is absorbed by almost all the substances (Koutchma et al. 2009). Food and Drug Administration (FDA) has approved the use of UV-C light in the processing of fruit juices to reduce the microbial load (USFDA 2000). UV-C light is well known as its germicidal effect on many microorganisms. Inactivation mechanism depends on the absorption of UV photons by the genetic materials and subsequently the formation of dimers, which inhibit the transcription, and replication of the cell. Firstly incident UV light penetrates the cell. Then, nucleic acids and their constituents absorb the light resulting in the formation of mutagenic and cytotoxic DNA lesions. These UV products are cyclobutane-pyrimidine dimers and 6-4

photoproducts. Primarily dimers cause the mutagenic effect by retarding and preventing the cell division due to the links between adjacent pyrimidine molecules in DNA. Therefore microorganisms become inactive and unable to cause infection (Oguma et al. 2002; Bolton and Linden 2003; Donahue et al. 2004; Koutchma 2009; Silva et al. 2003; Koutchma et al. 2009; Ou et al. 2011). Fredericks et al. (2011) applied UV-C treatment for unclarified Chenin blanc juice contaminated with *Brettanomyces bruxellensis* ISA 1649 and *Saccharomyces cerevisiae* VIN13 and reported 1.83 and 5.38 $\log_{10}$ reduction for these yeasts after a UV-C dosage of 1,377 mJ/mL, respectively (Fredericks et al. 2011). Keyser et al. (2008) applied 230 mJ/mL of UV in order to achieve 5.1 $\log_{10}$ reduction in apple juice samples inoculated with *E. coli* K12. 1.45 $\log_{10}$ reduction was observed in yeast and mould count in the pomegranate juice after receiving 62,4 J/mL UV dose (Pala and Toklucu 2011). In another study, *E. coli* ATCC 35218 in apple juice was decreased by 5.2 $\log_{10}$ after 15 min exposure to UV-C light whereas only 3.9 $\log_{10}$ reduction was achieved in the number of *S. cerevisiae* KE162 under the same treatment conditions (Char et al. 2010). Mukhopadhyay et al. (2011) showed that the yeast count in mango juice was reduced only 0.17 $\log_{10}$ by 60 min of UV-C light application. However, Engin et al. (2009) observed 1.5 $\log_{10}$ reduction in yeast and mould count in the UV treated milk samples applying light intensity of 0.26 mW/cm^2. Besides, UV treatment was also found to be effective on prolonging the shelf life of fruit and vegetables (Vunnam et al. 2012).

The objective of this study was to investigate the effect of UV-C irradiation by a collimated beam apparatus on the inactivation of spoilage microorganisms and colour of orange juice. It has been supposed that this processing technique will enhance the microbial safety of fresh squeezed orange juices without changing the colour of the product.

Materials and methods

Preparation of orange juice samples

Fresh oranges (Washington variety) were purchased from a local market in Izmir, Turkey. Prior to juice extraction oranges were washed and cut into half and pressed by using a household tabletop citrus juice extractor (Robolio, Arcelik, Turkey). In order to prevent contamination extracted juice was kept in the sterilized flasks. After extraction the microbial load of the samples was not detectable. Therefore prior to UV-C treatment, freshly squeezed orange juice samples were fermented to increase the number of microorganisms. Fermentation was performed under continuous shaking at 200 rpm using an incubator shaker (Thermo Scientific, Forma 481, USA) at 22 °C for 3 days. This led the microbial count to reach about 10^5–10^6 CFU/mL. The incubated orange juice was processed by UV-C irradiation.

Bench top UV-C irradiation equipment and microbial inactivation study

UV biodosimetry studies were performed in order to determine the microbial inactivation of orange juice by UV-C irradiation. Orange juice samples were exposed to UV-C irradiation using a collimated beam apparatus as described by Bolton and Linden (2003) (Fig. 1). Details of the system were given in Unluturk et al. (2008). Samples were placed in petri dishes directly below the collimated UV beam and stirred continuously during irradiation with a stirrer (IKA Yellow Line, Belgium) set at 200 rpm. The UV intensity at the surface of the sample (incident intensity (I_0) or irradiance at the surface) was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA). The radiometer was placed at a similar distance as the orange juice samples. The UV lamp was switched on for about 30 min prior to UV treatment of orange juice samples to reduce fluctuations in UV light. UV incident light intensity was measured as 1.32-mW/cm^2. Before usage, the collimated beam apparatus was cleaned and sanitized with 70 % (v/v) ethanol.

Orange juice samples (approx. 3 mL) were added to 50 mm standard petri dishes to obtain a sample depth of 0.153 cm. The depth was calculated from the ratio of the sample volume and the surface area of a petri dish. An adequate stirring was applied during treatment in order to ensure equal distribution of UV dose through the sample. The edge effects caused by stirring are avoided by using the smallest possible sample volume (Bolton and Linden 2003).

Biodosimetric study of endogenous microorganisms was carried out by direct UV light, exposed on 0.153 cm sample depth at a 1.32 mW/cm^2 UV incident intensity level for 3, 6, 9, 12, 15, 18 and 20 min except untreated control sample. All studies were conducted within the UV dose range, calculated by the product of incident intensity and exposure time, of 0 –

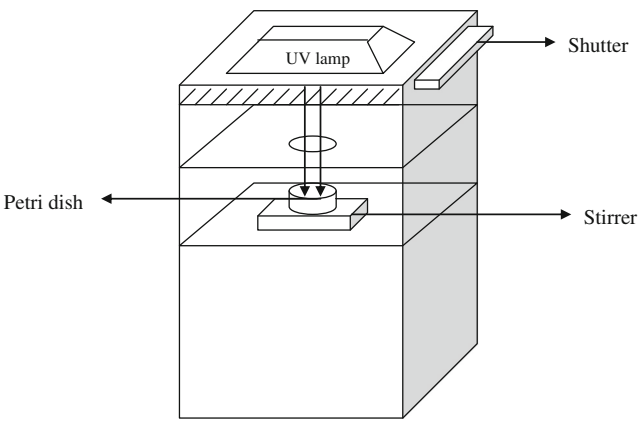

Fig. 1 Closed bench top UV system

108.42 mJ/cm^2. The calculation method of UV dose is described in detail by Unluturk et al. (2008). After the UV light exposure, orange juice samples were diluted with 0.1 % (w/v) sterile buffered peptone water (Merck, Darmstadt, Germany). Total aerobic microorganisms and yeast and mould counts were enumerated using spread plating method on Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) and Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) acidified with 10 % tartaric acid (Merck, Darmstadt, Germany). TSA plates were incubated at 37 °C for 24 h and counted. PDA plates were incubated at 25 °C for 5 days. Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) and Eosin Methylene Blue (EMB, Merck, Darmstadt, Germany) Agar media were utilized to check whether the test samples included coliform organisms. Plates were incubated at 37 °C for 24 h. All the measurements were replicated three times.

Measurement of physical and chemical properties

pH values of the samples were measured by using a bench top pH meter (HANNA Instruments, USA) at 20 °C. Mettler-Toledo RE40D Bench top Refractometer (AEA Investors Inc., U.S.A.) was used to determine the brix of the juice samples at 20 °C. Titratable acidity of the juice samples was determined using the method given by Cemeroglu (2007). Juice sample was titrated against standardized 0.1 N NaOH up to pH 8.1. The acidity of the sample was calculated according to the following formula given in Eq. (1). The most common acid in citrus juices is citric acid. Thus, results were expressed as grams of citric acid per 100 mL of fruit juice.

$$TA, \% = \frac{V \times f \times E \times 100}{M} \quad (1)$$

V; represents the volume of 0.1 N NaOH used up during the titration (mL), f; is the normality factor, E; is the milliequivalent weight of citric acid (g), M; is the volume of the sample (mL).

Measurement of optical properties

Absorbance measurements of the juice sample were performed using Cary 100 UV-Visible Spectrophotometer (Varian, USA) at 254 nm. Absorbance coefficient was calculated at the end of fermentation by measuring the absorbance of different dilutions of the juice and determined from the slope of absorbance versus concentration (Caminiti et al. 2010). HACH 2100AN IS Turbidimeter was used to find out the turbidity value of juices. The results were given in Nephelometric Turbidity Unit (NTU). Colour parameters of juice samples were determined by means of Konica Minolta CR 400 chromameter (Konica Inc. Japan). CIE L* (brightness-darkness), a* (redness-greenness), b* (yellowness-blueness) values were obtained before and after the UV exposure. Total colour difference (ΔE) was calculated according to Eq. (2). The data were analyzed by using Analysis of Variance.

$$\Delta E = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2} \quad (2)$$

Statistical analysis

Data analysis for the inactivation of spoilage microorganisms in fermented orange juice was performed by Design Expert 7.0.0 Trial Version (Stat-Ease Inc., USA) and Minitab 14.1 (Minitab Inc, US/Canada). The factor was exposure time and the response was log survival number. Effect of exposure time on the response was investigated by one-way ANOVA technique. Thus a one-factor design with 8 levels was adapted. Totally 24 runs were obtained. Also, Tukey's test was applied to the data in order to determine which level of the factor caused significant changes on the response.

Modelling the survival data

SigmaPlot 2,000 Version 6.00 (Chicago, IL, USA) was used for linear and non-linear regression analysis and to determine the parameters of the models. The goodness-of-fit of the models was assessed using adjusted determination coefficient (R^2_{adj}) and mean square error (MSE) values.

Results and discussion

Physical, chemical and optical properties of orange juice

Physical and chemical properties such as pH, Brix (soluble solids), titratable acidity, turbidity and colour values (L*, a*, b*) of the freshly squeezed orange juice are shown in Table 1.

Absorbance coefficient of freshly squeezed and fermented orange juice was determined at the wavelength of 254 nm as 71.715 and 95.498 cm^{-1}, respectively. Apple juice absorbance coefficient was reported as 5.81 and 6.11 cm^{-1} in different studies (Caminiti et al. 2010; Char et al. 2010). In another study, apple juice absorbance coefficient was reported as 19.81 cm^{-1} whereas fresh apple cider was found to have an absorbance coefficient as high as 25.8 cm^{-1} and turbidity levels of 600–1,600 NTU (Koutchma et al. 2004). The efficiency of UV light absorption by bacteria was reported to be reduced by the particles that can absorb scatter and block the UV light (Koutchma et al. 2004). The turbidity of freshly squeezed and fermented orange juice was similar and measured as 4061±43.73 NTU indicating that orange juice had significantly higher amount of suspended particles than apple cider. The presence of large suspended particles

Table 1 Physical properties of the orange juice

pH	Brix (%)	TA[a]	Turbidity	L*	a*	b*
3.67±0.014	13.74 ±0.223	1.398±0.019	4061±43.73 NTU	38.81 ±0.075	−2,67±0.026	22.72 ±0.102

[a] Total acidity in g Citric acid/100 mL

was demonstrated to cause a decrease in the UV penetration by increasing the UV absorptivity (Begum et al. 2009).

Microbial inactivation and modelling of survival curve

Results showed that fresh orange juice samples did not contain coliform bacteria. Initial total aerobic and yeast and mould counts were found as 5.19-log (±0.56) and 6.04-log (±0.38) CFU/mL respectively after incubation which were found to be statistically equal to each other at a significance level of 0.05. According to the simple staining and microscopic examination of the fermented orange juice, microbial population was found to be mainly composed of yeasts. Therefore, total yeasts and moulds counts were assessed in the course of the whole study. It was also indicated that yeasts are the major microorganisms found in the natural flora of orange juice (Molinari et al. 2004).

It was reported that initial microbial concentrations of 10^6 CFU/mL or less were found to be appropriate to observe a required reduction in the microbial population (Tahiri et al. 2006). In another study, the number of yeast cells in maple syrup greater than 10^5 CFU/mL caused an increase in UV absorbance (Murakami et al. 2006). As the absorbance values increase the UV-C light effectiveness is reduced therefore, in order to prevent the reduction of inactivation effectiveness and to observe required reduction, incubation was terminated when the yeast count reached to 6.04-log (±0.38) CFU/mL.

Other than initial load, ANOVA analysis indicated that the exposure time also has a significant effect on the inactivation of natural flora of the orange juice ($p<0.0001$). Tukey's test showed that increasing the UV exposure time resulted in significantly lower log survival numbers (data not shown).

The maximum log reduction in yeast and mould count was obtained as 1.76 $\log_{10}$ after 20 min of UV exposure (I_0= 1.32 mW/cm^2) at a UV dose of 108.42 mJ/cm^2. Tran and Farid (2004) reported almost 0.39-log reduction in total yeast and mould count in reconstituted orange juice after treatment with UV dose of 123 mJ/cm^2. However, almost 0.34-log reduction in total yeast and mould count was observed in the same study for fresh squeezed orange juice treated with UV dose of 73.8 mJ/cm^2. In another study a 0.41-log reduction in total yeast and mould count was observed by treating the orange juice in a continuous system at a UV dose of 125 mJ/cm^2 (Torkamani and Niakousari 2011).

Char et al. (2010) demonstrated that UV-C treatment provided lower inactivation for *S. cerevisiae* than *E. coli* ATCC 35218. UV light inactivation was found as not effective as thermal pasteurization on the yeast and mould populations (Tandon et al. 2003). Similar findings were obtained in this study. Yeasts and moulds are more resistant to ultraviolet light than bacteria (Tran and Farid 2004). This is due to their big sizes, less pyrimidine base content of their genetic material, differences in their cell wall compositions and thickness. For example, the cell size of *S. cerevisiae*, *E. coli* and *L. innocua* were reported as 8–10 μm (in diameter), 1×3 μm and 0.5×1.5 μm, respectively (Guerrero-Beltran et al. 2011). Also rich growth medium was reported to increase the number of ribosomes that provide a shield for the DNA against UV light (Tran and Farid 2004). Additionally, it was revealed that inactivation of natural flora is more difficult compared to the inactivation of inoculated microorganisms. Besides, the existence of UV absorptive materials, like suspended particles and yeasts, was demonstrated to reduce the effectiveness of UV radiation (Oteiza et al. 2010). They increase the necessary dose to deactivate target micro-organisms (Oteiza et al. 2010). Orange juice was reported to need higher UV doses in order to reach a satisfactory reduction level while lower doses are sufficient for clear juices (Keyser et al. 2008).

The inactivation of microorganisms by heat and other processing methods has been traditionally assumed to follow first-order kinetics. There is now enough evidence that thermal inactivation of microorganisms does not follow first-order kinetics and the same can be said about non-thermal inactivation methods such as high hydrostatic pressure (HHP), pulsed electric fields (PEF), UV-C irradiation (Buzrul and Alpas 2004; Chen 2007; Bermúdez-Aguirre and Corradini 2011; Unluturk et al. 2010).

Over the years, several models have been proposed to describe the non-linear survival curves such as the ones described by Cerf (1977), Bhaduri et al. (1991), Cole et al. (1993) and Peleg and Cole (1998). Among the non-linear models, perhaps, the most simple and flexible one is the Weibull model (Eq. (3)) that is gaining popularity:

$$\log_{10} S(t) = -\left(\frac{t}{\delta}\right)^n \tag{3}$$

where $S(t)$ is the survival ratio i.e., $S(t)=N(t)/N_0$, [$N(t)$ and N_0 are the number of survivors after an exposure time t and

initial number of micro-organisms (CFU/mL), respectively]. This model is characterized by two parameters; δ is called time of first decimal reduction (units in min or s) i.e., time needed to reduce the initial population, N_0 to $N_0/10$ and n is the shape parameter.

The main advantage of this model is its simplicity and robustness to describe both monotonic downward concave (shoulder) survival curves ($n>1$) and monotonic upward concave (tailing) survival curves ($n<1$). Traditional first-order model Eq. (4) can be derived from the Weibull model and corresponds to a special case ($n=1$) of the Weibull model:

$$\log_{10} S(t) = -\frac{t}{D} \quad (4)$$

where D, is the decimal reduction time; time required for one log reduction in number of cells.

It was observed that survival curve indicated a concavity (Fig. 2). Therefore Weibull model was assumed to best describe the survival data. Fig. 2 also shows the fit of the Weibull and the traditional first-order models. It can be seen that traditional first-order model is inadequate; however, the Weibull model with one more parameter could be successfully used to describe the data. Support for this statement comes from higher adjusted determination coefficient (R^2_{adj}) and lower mean square error (MSE) values obtained for the Weibull model (Table 2).

According to Table 2, time and UV dose of first decimal reduction were obtained as 5.7 min and 31 mJ/cm^2, respectively. Tran and Farid (2004) reported the decimal reduction constant as 119±17 mJ/cm^2 for yeasts and moulds in reconstituted orange juice (10.58 Brix). In another study D_{10} value for yeast and mould in orange juice was found as 105 mJ/cm^2 (Torkamani and Niakousari 2011). Additionally it was revealed that *Saccharomyces cerevisiae* had the least resistance (D_{10}=6.38 min) to UV-C light in clear apple juice among the tested yeast species (Gabriel 2012). According to results D_{10} value for this study is less than those of given in literature. The shape of the inactivation curve could explain the reason for this.

Shape parameter of the Weibull model was less than one ($n<1$ i.e., tailing or upward concavity) indicating that remaining members of the population have the ability to adapt to applied UV-C irradiation (Van Boekel 2002). Therefore, it can be interpreted as evidence that sensitive members of the populations are destroyed at a relatively fast rate leaving behind the survivors of higher and higher resistance (Bermúdez-Aguirre and Corradini 2011). Nevertheless, it should be noted that inactivation was only about 2 $\log_{10}$ (Fig. 2) and this may not reflect real survival pattern (initial number of the microflora was about 6 $\log_{10}$) (Table 2).

Effect of UV on orange juice colour

The effect of UV-C irradiation on the colour of orange juice was investigated in this study. Fig. 3a, b, c indicate that a^* and b^* parameters showed slight changes after the UV exposure, whereas L^* value remained almost constant. Orange juice is a highly pigmented product due to its high carotenoid content. In literature, it was reported that highly pigmented juices are less affected by the processing and storage (Lee and Coates 1999). High concentrations of colour pigments provide a better masking effect on colour differences. These type of juices have more acceptible colour after the processing (Lee

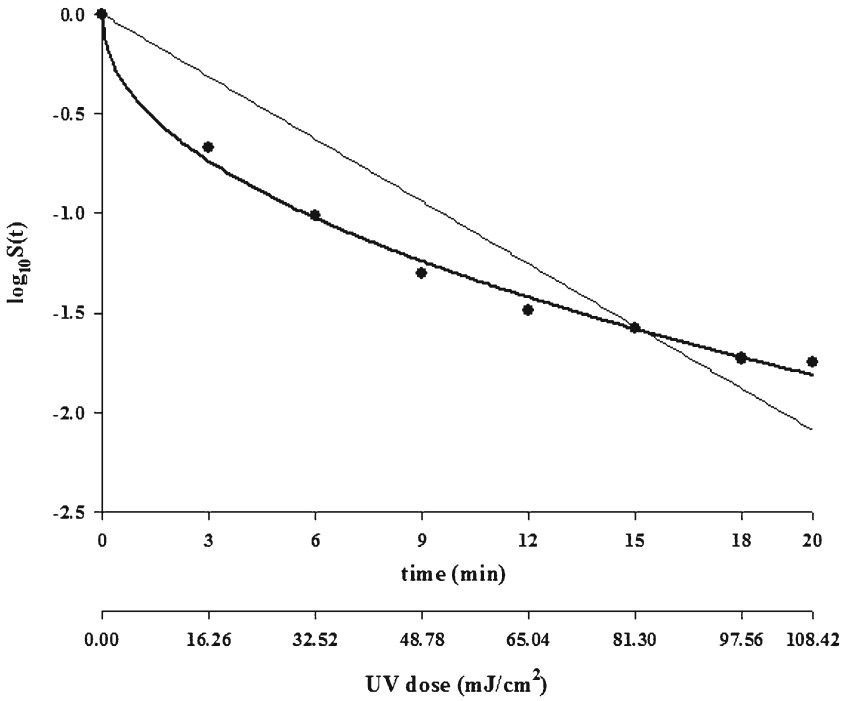

Fig. 2 Survival curve of natural microflora in orange juice exposed to UV-irradiation (time in minutes, UV dose in mJ/cm^2). Thick line indicates data were fitted with the Weibull model [Eq. (3)] and thin line indicates data were fitted with the linear model [Eq. (4)]. Data were the average of three replicates

Table 2 Estimated parameters of the Weibull and linear models±standard deviation

Weibull model [Eq.(3)]					Linear model [Eq.(4)]			
δ (minute)	δ (mJ/cm^2)	n (−)	R^2_{adj} [a]	MSE[b]	D (minute)	D (mJ/cm^2)	R^2_{adj} [a]	MSE[b]
5.7±0.4	30.99±1.93	0.48±0.03	0.99	0.003	9.6±0.8	51.83±4.15	0.77	0.09

[a] Adjusted determination coefficient

[b] Mean square error

and Coates 1999). Some changes were obtained for ΔE values after UV-C light irradiaiton but no clear trend was observed (Fig. 3d). ANOVA results showed that UV exposure time was not a statistically significant factor affecting the colour parameters (L^*, a^*, b^*) and total colour (ΔE) of the orange juice within the selected levels ($p>0.05$).

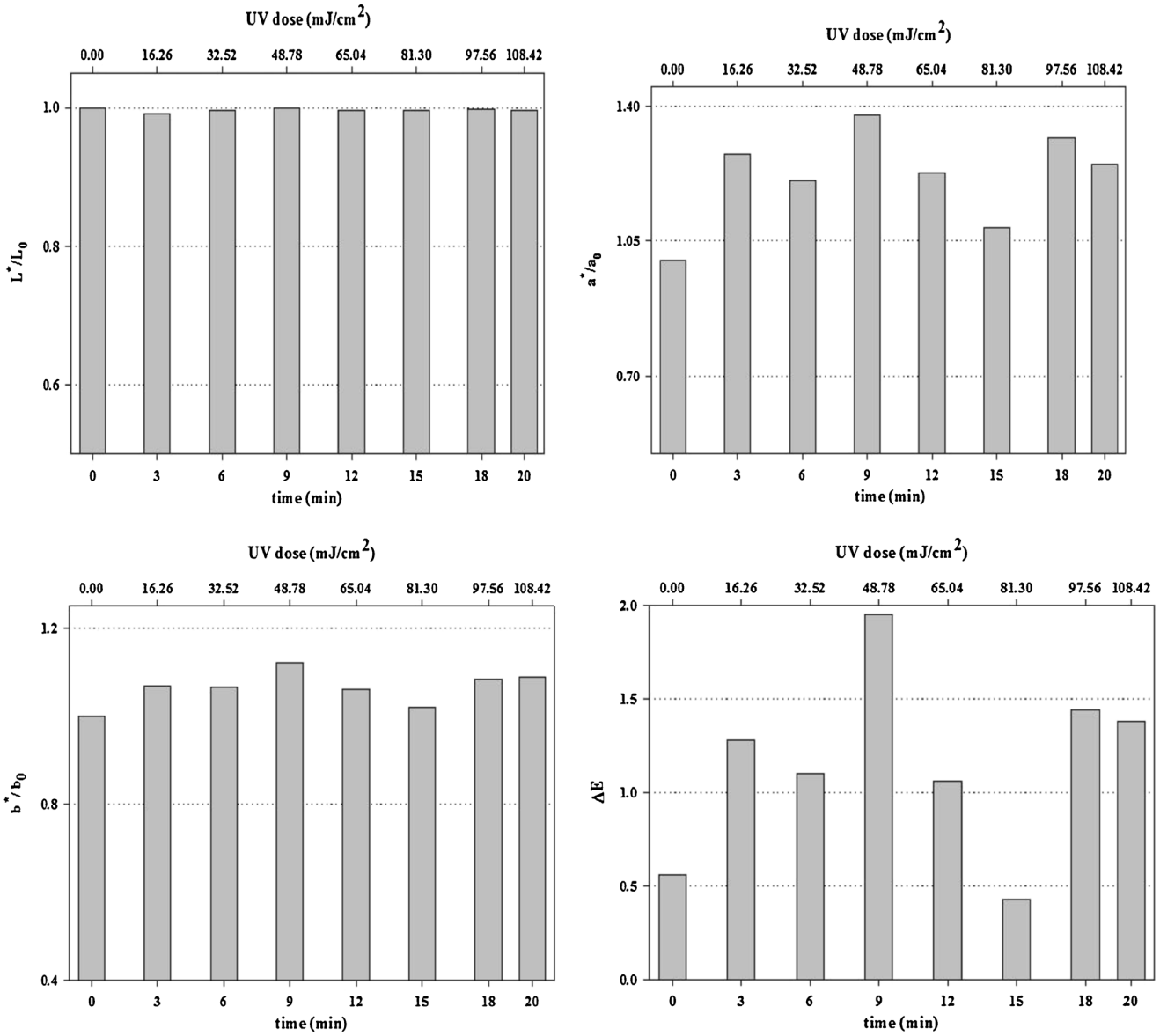

Fig. 3 Relative changes in colour parameters and total colour after UV-C irradiation treatment. L^*/L_0, a^*/a_0, b^*/b_0 and ΔE in function of exposure time in minutes and UV dose in mJ/cm^2

Conclusions

It was concluded that the resistance of natural flora and existence of suspended matters limited the effectiveness of the UV-C irradiation process. The spoilage microorgansims (i.e. yeasts) in their natural environment exhibit higher resistance to UV irradiation than inoculated microorganisms (e.g. *Saccharomyces cerevisiae*). The data suggests that biodosimetric studies performed by using inoculated microorganisms for assesment of the efficiency of UV irradiation treatment in the the shelf life extension of juices must be carefully evaluated. The UV exposure time was found to be a very important ($p<0.0001$) factor on log survival number, the usage of UV-C irradiation showed a good potential achieve a required level of microbial reduction in orange juice. Additionally, the colour of the juice was not influenced by UV-C irradiation.

Inactivation of naturally grown microorganisms using UV-C light can be investigated by combining this technology with other non-thermal processes in a hurdle strategy to increase the effectiveness of this technology additionally and extend the shelf life of fresh orange juice.

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