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Effect of ultraviolet-C light and mild thermal treatment on the storage life of orange juice

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Abstract: This study aimed to evaluate the effect of low-dosage treatment with ultraviolet-C light (19.75 J L^{-1} for 5 min), mild thermal treatment (40 °C for 5 min), or their combination on the native microflora (mesophiles and moulds plus yeast) and consumer acceptance of orange juice at the beginning and after storage (5 or 22 °C). Results indicated that UV-C light and mild thermal treatments reduce 0.19 ± 0.03 and 0.25 ± 0.02 log cycles (both kinds of microorganisms), respectively. The combination of treatments displays an additive effect against mesophiles (0.47 ± 0.01 log) and moulds plus yeasts (0.42 ± 0.02 log). After 9 days of storage at 5 °C, combined treatment did not present any microbial increases ($P > 0.05$), while consumer acceptance was similar ($P > 0.05$) to the fresh orange juice. Although several studies about the use of hurdle technology using UV-C light in orange juice have to be conducted, the results obtained in this study are promising, and they can be used for further studies.

Keywords: hurdle technology; native microflora; storage life; liquid food; UV-C light; minimal processing

Orange juice is the most popular fruit juice worldwide. Its global production has been increasing (23% higher than in 2018) in recent years (USDA 2019). The higher demand for orange juice is due to its sensory and nutritional characteristics (Velázquez-Estrada et al. 2019). In this aspect, although fresh orange juice is preferred by the consumers, different outbreaks of infection have been associated with the consumption of fresh fruit juices (Gabriel et al. 2016); therefore, they have to be pasteurised for their safe consumption. Thermal treatment is the most commonly used technology to pasteurise fruit juices; however, it is well-known that this technology affects their desired quality attributes (Lo Scalzo et al. 2004; Sentandreu et al. 2005).

Ultraviolet-C light is a technology widely used for water and surface disinfection (Koutchma et al. 2016). However, it is currently applied for treating juices, nectars, and beverages (Koutchma et al. 2016; Shah et al. 2016) because UV-C light damages the DNA of microorganisms, which affects their replication, conducing to the cell death (Ochoa-Velasco et al. 2018). Nevertheless, one of the main problems of the UV-C light treatment is the reactivation process, presented when the inactivation of the microorganisms was incomplete and the storage conditions are inadequate

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(Torkamani and Niakousari 2011; Beristaín-Bauza et al. 2018). Therefore, the use of combined treatments or hurdle technology to reduce and/or maintain the microbial load during the storage of UV-C treated foodstuff is necessary (Pala and Toklucu 2013; Beristaín-Bauza et al. 2018). In this aspect, different studies about the use of UV-C light plus other technologies for treating orange juice have been conducted. For example, Gayán et al. (2012) showed an *Escherichia coli* reduction higher than 5-log cycles with the combination of UV-C light treatment (27.10 J mL^{-1}) and heat (55 °C). While Pala and Toklucu (2013) applied a high UV-C light dose (36.09 kJ L^{-1}) to orange juice and its effect was evaluated during the refrigeration storage; their results indicated that after 14 days of storage, the total aerobics and moulds plus yeasts increased by almost one and three log cycles, respectively. Then, a constant microbial load was reported. However, to our knowledge, the effect of the low-dose of UV-C light plus mild thermal treatment to increase the storage life of orange juice has not been reported yet.

Thus, the aim of this research was to evaluate the effect of UV-C light, mild thermal treatment and their combination on the native flora and consumer acceptance of fresh and stored orange juices.

MATERIAL AND METHODS

Orange juice. Oranges (*Citrus sinensis* L.) were purchased from a local supermarket (Puebla, Mexico). Fruits free from appearance damage were selected, washed, disinfected with sodium hypochlorite (150 mg L^{-1}), and dried with absorbent paper. Fruits were manually cut, squeezed, filtered (800 μm), and immediately used for processing.

UV-C light and thermal equipment. The UV-C light plus heat treatment equipment was designed and assembled in the Facultad de Ciencias Quimicas of Benemerita Universidad Autonoma de Puebla (Puebla, Mexico). The equipment consists of one UV-C light lamp (TodoAgua, Mexico), a double-wall reactor, one feeding tank, one heating tank, two peristaltic pumps, and a reception tank. The double-wall reactor (Figure 1) consists of (a) a UV-C light lamp, (b) helically coiled with a crystal tubing, where the orange juice is pumped, and (c) a heating chamber, where the hot water circulates to reach the temperature of processing. For processing, orange juice flows into the tube while hot water circulates inside the chamber. The temperature of the chamber was measured inside the double wall-reactor, and the irradiation dosage was evaluated by the actinometric methodology (Rahn 1997). To ensure the process temperature, it has to be higher (5 °C) than the temperature inside the double-wall reactor.

UV-C and thermal processes. Four orange juice batches were used in this study. Treatment 1 was untreated orange juice (control), treatment 2 consisted of orange juice treated with UV-C light (19.75 J L^{-1} for 5 min), treatment 3 consisted of mild thermal treated (40 °C for 5 min, UV-C light lamp turned off) orange juice, and treatment 4 was orange juice treated with both UV-C light (19.75 J L^{-1}) plus mild thermal treat-

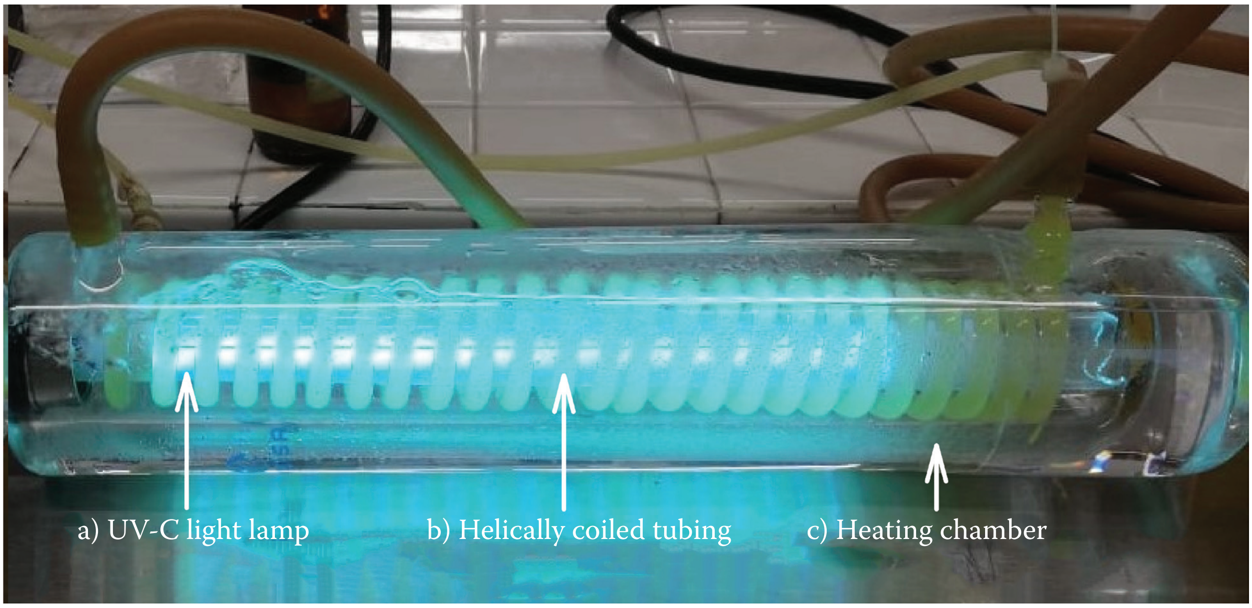

Figure 1. Double-wall UV-C light reactor

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ment (40 °C) for 5 min. To conduct each experiment, 100 mL of orange juice were placed into the feeding tank and when the condition was stable, the fluids circulated throughout the equipment at 0.44 mL s^{-1}. The flow rate was calculated based on an assumption that orange juice can remain 5 min in the UV-C light tubing. The sample was taken after one passage through the equipment (5 min) and stored in sterile glass bottles at refrigeration (5 ± 1 °C) or room temperature (22 ± 3 °C).

Microbiological analysis. One millilitre of orange juice or diluted (by peptone water) orange juice was placed on standard plate count agar (BD Bioxon, Mexico) and acidified potato dextrose agar (BD Bioxon, Mexico) to count the mesophiles and moulds plus yeasts (30–300 CFU mL^{-1}), respectively (Reasoner 2004). The microbial counts were done after 24 h (30 ± 2 °C) for mesophiles and 72 h (25 ± 2 °C) for moulds plus yeasts. Results were expressed as log [N (CFU mL^{-1})/N_0 (CFU mL^{-1})].

Microbial growth kinetics. The effect of treatments during orange juice storage was evaluated using the Weibull model (Equation 1):

$$log\left(\frac{N}{N_0}\right) = bt^n \quad (1)$$

where: N_0 and N is the microbial load at the beginning and after a given storage time, respectively, t is the storage time, and b (inactivation rate) and n (resistance of the microorganisms to the storage condition) are the Weibull model parameters. If n is equal to 1, the Weibull model becomes a first-order model, while if n is lower than 1 (concave curves), the microorganisms become more resistant to the storage condition having the ability to adapt to it, and if n is higher than 1 (convex curves), the microorganisms become more sensitive to the storage condition.

Consumer acceptance evaluation. Fresh, treated and stored (juice with the lower microbial load) orange juices were evaluated for their general acceptance by using a 9-point hedonic scale, where: 1 = dislike very much and 9 = like very much (Wichchukit and O'Mahony 2015). To conduct the test, 10 mL of tempered (at room temperature) orange juice were provided to 100 untrained judges (18–25 years old) who frequently consume orange juice.

Statistical analysis. All determinations were done in duplicate and each experiment was performed in triplicate. The KaleidaGraph 3.51 program was used to fit the experimental data to the Weibull model. Statistical analysis was performed by analysis of variance using the Minitab 16 program (Minitab Inc., USA). Tukey's test (α = 0.05) was used for deciding significant differences between averages of treatments.

RESULTS AND DISCUSSION

Inactivation of native microorganisms in orange juice by UV-C light, thermal treatment and their combination. The effect of UV-C light treatment, mild thermal treatment and their combination on the mesophiles (A) and moulds plus yeasts (B) in orange juice is shown in Figure 2. As observed, in both types of microorganisms, the mild thermal treatment (40 °C for 5 min) exhibited a higher ($P < 0.05$) microbial reduction (0.25 ± 0.02 log) than those results (0.19 ± 0.03 log) obtained with the UV-C light treatment (19.75 J L^{-1}). It is well-known that the UV-C light efficacy on microbial reduction is widely affected by some intrinsic factors of the liquid products such as soluble and insol-

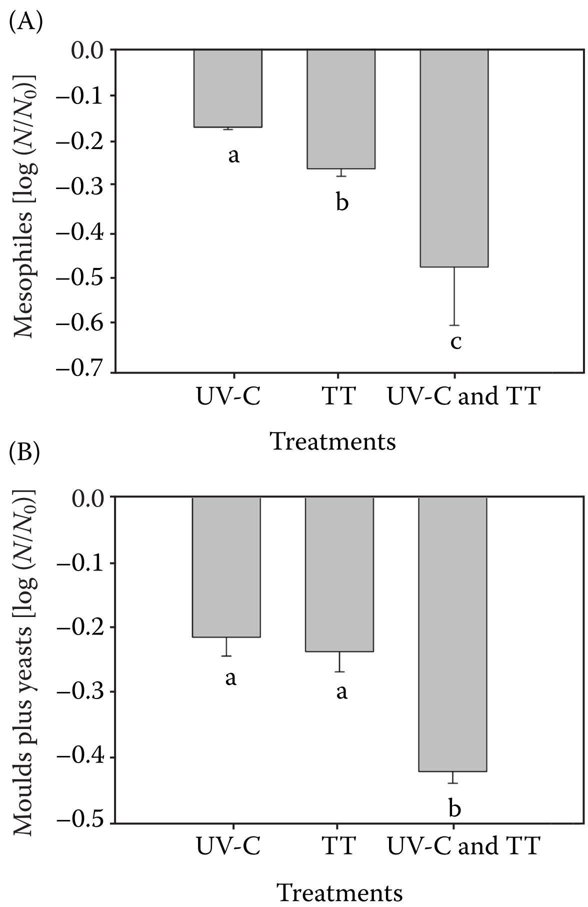

Figure 2. Mesophiles (A) and moulds plus yeasts (B) of orange juices treated with UV-C light, thermal treatment (TT) and their combination

Bars indicate standard deviation (SD)

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uble solids, absorption coefficient, colour, etc. (Guerrero-Beltrán et al. 2009). On the other hand, the thermal treatment may produce a phase transition of the cell wall phospholipid bilayer from gel to liquid crystalline, leading to microbial inactivation (Gayán et al. 2011). The combined treatment (19.75 J L^{-1} and 40 °C) showed an additive effect because a microbial reduction of 0.47 ± 0.01 log and 0.42 ± 0.02 log was obtained for mesophiles and moulds plus yeasts, respectively. The results obtained in this study are similar to those attained by Gayán et al. (2012), who used a combined treatment of a high dose of UV-C light (15–20 J mL^{-1}) and mild thermal treatment (40 °C) for treating orange juice inoculated with *E. coli* ATCC 4201. They pointed out that the application of UV-C light alone was not enough to reach more than one log cycle of the microorganism tested, but, as the temperature increased, a synergistic effect of UV-C light and heat was observed. Although the microbial reduction was low and did not attain the 5-log reduction required by the FDA (2001), it is noteworthy that native microflora is more resistant to the UV-C light treatment than specific microorganisms; therefore, a higher UV-C light dosage is required to reach the same inactivation (Pala and Toklucu 2013). However, the use of low energy cost treatments or hurdle technology is currently on the rise (Khan et al. 2017).

Microbial growth in orange juice during storage. One of the main purposes of this study was to evaluate the effect of UV-C light treatment, mild thermal treatment and their combination on the microbial stability of stored orange juice. Figure 3 shows the microbial behaviour of treated and untreated orange juice during storage. Orange juice stored at room temperature (Figures 3A and 3B) showed an increase in both kinds of microorganisms, regardless it was treated or not, reaching a microbial load after 15 days of storage of 6.54 ± 0.16 and 4.27 ± 0.46 log for mesophiles and

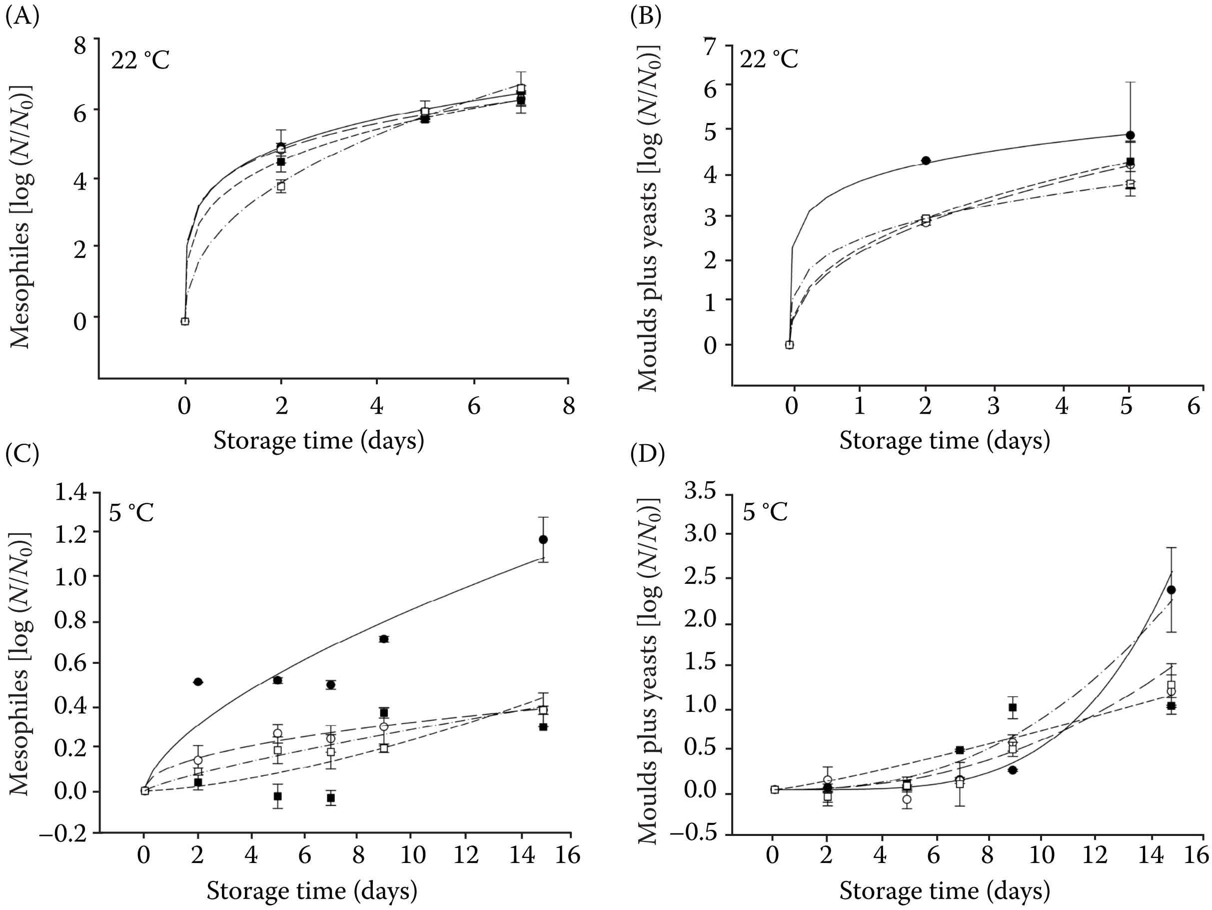

Figure 3. Mesophiles (A and C) and moulds plus yeasts (B and D) of untreated orange juices (●), treated with UV-C light (○), thermal treatment (■) and treated with their combination (□)

Dots and lines are experimental and fitted data, respectively; bars indicate SD

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Table 1. Weibull parameters of microbial growth of treated and untreated orange juices

Treatment	Mesophiles			Moulds plus yeasts		
	b	*n*	R^2	*b*	*n*	R^2
5 °C						
Fresh	$0.20 \pm 0.05_a$	$0.63 \pm 0.13_{ab}$	0.88	$4.2 \times 10^{-5} \pm 2.53 \times 10^{-5}{}_b$	$4.07 \pm 0.32_a$	1.00
UV-C light	$0.11 \pm 0.02_{ab}$	$0.46 \pm 0.08_b$	0.98	$3.1 \times 10^{-3} \pm 2.5 \times 10^{-3}{}_b$	$2.27 \pm 0.40_b$	0.91
TT	$0.01 \pm 0.01_b$	$1.51 \pm 0.39_a$	0.53	$0.05 \pm 0.005_a$	$1.17 \pm 0.05_b$	0.81
UV-C and TT	$0.05 \pm 0.03_b$	$0.79 \pm 0.20_{ab}$	0.95	$2.8 \times 10^{-3} \pm 3.3 \times 10^{-3}{}_b$	$2.47 \pm 0.6_b$	0.98
22 °C						
Fresh	$4.34 \pm 0.3_a$	$0.21 \pm 0.02_a$	1.00	$3.79 \pm 0.49_a$	$0.22 \pm 0.15_a$	1.00
UV-C light	$4.32 \pm 0.4_a$	$0.20 \pm 0.06_a$	1.00	$2.13 \pm 0.13_b$	$0.42 \pm 0.12_a$	1.00
TT	$3.88 \pm 0.5_a$	$0.26 \pm 0.08_a$	1.00	$2.23 \pm 0.14_b$	$0.40 \pm 0.11_a$	1.00
UV-C and TT	$2.98 \pm 0.3_a$	$0.43 \pm 0.05_a$	1.00	$2.45 \pm 0.12_b$	$0.26 \pm 0.08_a$	1.00

b – inactivation rate; *n* – resistance of the microorganisms to the storage condition; *b* and *n* are shown as mean ± SD; R^2 – coefficient of determination; TT – thermal treatment; values with different letters within the same column are statistically different ($P < 0.05$)

moulds plus yeasts, respectively. Thus, the storage condition is very important to enhance the shelf life of orange juice. In this sense, orange juice stored under refrigeration conditions showed different behaviours. Untreated orange juice showed the mesophiles increase (Figure 3C) since the first day of storage, reaching 1.18 ± 0.11 log cycles after 15 days of storage. While in treated orange juices, mesophiles slightly increased during storage, reaching a maximum microbial load of 0.35 ± 0.02 log cycles (< 360 CFU mL^{-1}) in orange juice treated with the hurdle technology. On the other hand, moulds plus yeasts remained constant for almost 7 days of storage. The orange juice treated with the hurdle technology did not present any significant microbial increases ($P > 0.05$) during 9 days of storage, showing a microbial load lower than 0.48 log cycles (< 100 CFU mL^{-1}). In this aspect, some studies have indicated that the application of UV-C light alone was not enough to maintain the microbial load during storage due to if a complete inactivation is not attained, photoreactivation or dark reparation processes may occur; thus, the combination of treatments is recommended to maintain the microbial load of juice during storage (Beristaín-Bauza et al. 2018; Ochoa-Velasco et al. 2018). The results obtained in this study are similar to those obtained by Torkamani and Niakousari (2011) and Pala and Toklucu (2013). They used UV-C light for treating orange juice and pointed out that orange juice treated with a high dose of UV-C light showed a short storage life not longer than 14 days at 4 °C.

The Weibull model was used to fit the microbial growth behaviour in orange juice (Table 1). As observed, the microbial kinetics of almost all microorganisms displayed an acceptable fitting to the Weibull model ($R^2 > 0.80$); only the kinetics for mesophiles at 5 °C did not show an adequate adjustment (0.53) to the model employed. As mentioned before, the *b* and *n* parameters of the Weibull model indicate the activation rate and microbial behaviour during storage, respectively. In this aspect, the *b* parameter was always lower in treated orange juices compared to untreated orange juice, while the *n* parameter was lower than 1 in mesophiles (5 and 22 °C) and moulds plus yeasts (22 °C), indicating that the microorganisms become more resistant and they can adapt to storage conditions. On the other hand, moulds plus yeasts stored at 5 °C display an *n* value higher than 1, which indicates that the microorganisms become more sensitive to the storage condition. This is very important because the main problem in citric juices is the growth of moulds and/or yeasts (Hakguder et al. 2015).

Consumer acceptance. Figure 4 shows the consumer evaluation of fresh, treated and stored orange juices. As observed, all juices showed similar acceptance, ranging from slightly like to moderately like (6 to 7). In this

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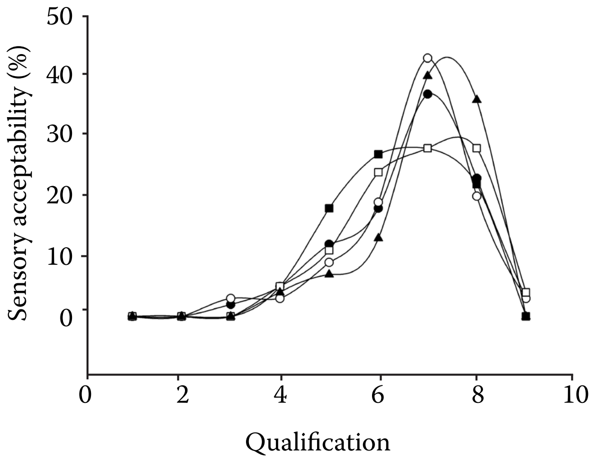

Figure 4. Consumer acceptance of fresh orange juice (●), after treatment with UV-C light (○), thermal treatment (■), their combination at the beginning of storage (□) and after 9 days (▲)

aspect, neither the individual treatment nor the combination of both treatments affects the consumer acceptance of the juices, which can be attributed to the fact that UV-C light and mild thermal treatment did not affect the physicochemical properties of orange juice (Gayán et al. 2012). Contrary to the results obtained in this study, Pala and Toklucu (2013) informed that the orange juice treated with UV-C light was less accepted by consumers, compared to fresh orange juice. However, the judges were not able to detect differences between both juices in the triangle test. On the other hand, orange juice treated with the hurdle technology and stored for 9 days displayed a slightly higher ($P > 0.05$) acceptance value (6.97 ± 1.07) compared to the fresh orange juice (6.64 ± 1.29). Therefore, the use of hurdle technology did not affect the consumer acceptance of orange juice, even after 9 days of storage.

CONCLUSION

The application of both UV-C light and mild thermal treatment produced a similar microbial reduction in the native microflora of orange juice, while their combination had an additive effect on microbial inactivation. After 9 days of storage, orange juice treated with hurdle technology did not present any microbial increases ($P > 0.05$), showing good consumer acceptance. Although further studies have to be conducted about the use of UV-C light alone or in combination with other technologies, this study indicates that it can be used for increasing the storage life of orange juice, maintaining an adequate consumer acceptance.

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