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The effect of photosensitization mediated by curcumin on storage life of fresh date (*Phoenix dactylifera* L.) fruit

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Highlight

- Photosensitization-Curcumin reduced fungal occurrence on fresh date surface
- Photosensitization-Curcumin ~~can~~ extend**ed** the shelf life of **chilled fresh** date fruit **under retail market conditions**
- No changes in physio-chemical properties were observed in dates after treatment

ABSTRACT

The effect of photosensitization mediated by curcumin (CUR) in sanitizing the fresh date fruit surface was investigated. Approximately 3 – 4 µl CUR solution ranging from 1000 to 1800 µM was sprayed on the date surface, followed by irradiation using blue light at 420 nm for 10 and 15 min at light dosages of 180 J/cm^2 and 270 J/cm^2 respectively. After irradiation, the date was stored at 4˚C and 30˚C until fungi was visually observed. The combination of CUR and light resulted in significant ($p<0.05$) extension of the shelf life and quality of fresh date. The shelf life of date fruit photosensitized by 1400 µM CUR for 10 min and stored at 30˚C lasted for 14 days in comparison to the 7 days for the control, while date stored at 4˚C lasted 98 days for the photosensitized fruit compared to the control of 28 days under the same conditions. In addition, no changes in physico-chemical characteristics were observed after treatment. The sanitization treatment of photosensitization mediated by CUR indicated a promising strategy in extending the shelf life of fresh date fruit. Clean and green technologies that are environmentally friendly like photosensitization are preferred by consumers to the synthetic chemicals used in conventional practices for sanitizing food.

Key Words :

Photosensitization; CUR; date fruit; fungal contamination; storage

1. INTRODUCTION

Dates (*Phoenix dactylifera* L.) are one of the main crops cultivated in arid and semi-arid regions of the Middle East and North Africa. The annual world production of date was estimated to reach 7.6 million metric tons with an average yield of 6.5 tons/ha (FAOSTAT, 2014). Fungal contamination is one of the main factors causing fruit decay and quality deterioration resulting in post-harvest and economic losses. The contamination can occur during harvest, handling, transportation and marketing. According to Atia (2011), postharvest losses of dates caused by fungal contamination ranged from 25 to 50% of total production.

Fresh dates are prone to fungal contamination in the field, during harvest, transportation, and marketing and during use by the consumer. Using inappropriate postharvest practices can increase the incidence of fungal contamination. Fresh dates contaminated by a combination of microbes and in most circumstances, these microbes can adhere to the outer skin of dates prior to harvest and remain relatively inactive with no expression of symptoms. After harvest and during ripening, however, the defense function of unripe skin falling and the quiescent fungi begins to develop and cause decay. If a wound occurs on the skin, fungi will penetrate the fruit cuticle and epidermis and grow rapidly (Fleet, 1992). In fact, some fungi possess enzymes to degrade the skin and penetrate the fruit even if a wound does not occur (Guetsky et al., 2005).

Among maturation stages, *Khalal* and *Rutab* are considered the most susceptible stages for fungal infection, where high moisture content and sugar provide favorable substrate for microorganisms (Shenasi et al., 2002). Date infected by pathogenic fungi, which may be associated with mycotoxin production, while spoilage fungi can cause deterioration of fruit quality. Mycotoxigenic species such as *Aspergillus niger*, *A. flavus* and *Fusarium* spp. have

been frequently isolated from date (Al-Asmari et al., 2017b, Al Hazzani et al., 2014, Kader and Hussein, 2009). Therefore, the threat of mycotoxin contamination of human and animal foods is a global concern (Wagacha & Muthomi, 2008). A high consumption of date, particularly in Arabian regions lead us to explore an alternative, effective and affordable method for sanitizing date.

Sanitizing date surface is an essential practice to minimize postharvest microbial infection, thereby extending date quality and shelf life. Unfortunately, the current treatment of sanitizing date with chlorine water is insufficient to kill the microorganisms that occur on date (Aleid, 2013). Alternative strategies including high hydrostatic pressure treatment (Aleid, 2013), electrolyzed oxidized water (Aleid & Hamad, 2013), ozonation (Najafi and Khodaparast, 2009), low energy X-ray irradiation (Aleid, 2013) were carried out on dates to decontaminate microbes. However, the treatments could not be applied commercially due to their limitations.

Photosensitization as a novel treatment has shown a promising effect against a wide range of food related microorganisms. These would include bacteria (Marc et al., 2009, Murdoch et al., 2010, Romanova et al., 2003, Vilela et al., 2012) and fungi (Lukšiene et al., 2005, Dovigo et al., 2011, Gonzales et al., 2010, Luksiene, 2004, Mitoraj et al., 2007, Preub et al., 2014, . However, there is limited information on the use of photosensitization as a sanitizer in food applications. In the case of a food system, photosensitizer (PS) should be safe for human consumption, cost-effective and chemically stable. Curcumin (CUR) from *Curcuma longa* L. has been used as a food grade photosensitizer to reduce microbial populations and increase shelf life of maize kernels (Temba et al., 2016) and oyster (Liu et al., 2016).

The photochemical interaction between the non-toxic photosensitizer (CUR) and visible light at an appropriate wavelength is known as photosensitization. This interaction in the presence

of oxygen generates cytotoxic substances, which causes damage to microbial cells. It causes biochemical and functional disturbances of the cell membrane component, which can include cross-linking, protein photooxidation, lipid peroxidation, leakage of lysosomal enzymes, inhibition of transport of some essential metabolites and increase in PS uptake by cell membrane, consequently leading to cell death (Zerdin et al., 2009, Bertoloni et al., 1989).Photosensitization mediated by CUR (photosensitization-CUR) could replace the conventional chemical disinfection of food. Our previous study (Al-Asmari et al., 2017a) showed the potential effect of photosensitization-CUR *in vitro* in controlling fungal spores that could be present in fresh dates. The present study aimed to investigate the effect of photosensitization-CUR *in vivo* to sanitize the surface of fresh date and extend the shelf life under different storage temperatures.

2. MATERIALS AND METHODS

2.1 Preparation of photosensitizer solution and irradiation source

CUR stock solution (2000 µM) was prepared according to Al-Asmari et al., 2017a by dissolving 73.8 mg CUR powder (Sigma Aldrich, St. Louis, USA) in 30 ml propylene glycol ($C_3H_8O_2$) and toped up to 100 ml using sterile water. Propylene glycol used as a solvent instead of ethanol as it is generally recognized as safe (GRAS) substance. This ratio of propylene glycol to water selected as preliminary studies showed no effect on spores (data not shown). The stock solution filtered through 0.20 µm filter and kept in a cool, dark place. From the stock solution, desired serial dilutions (1800, 1400 and 1000 µM, pH 5.3) were prepared. The irradiation conducted using a 500-Watt Xenon arc lamp (Polilight, PL 500) equipped with an optical fiber light guide over a range of 370 - 680 nm. The maximum absorbance wavelength (λ_{max}) of CUR to activate CUR molecules found to be 420nm from

the absorption spectrum measured using a spectrophotometer (Tecan, Infinite M200, Austria).

2.2 *In vivo* photosensitizing date fruits

The cultivar of *Barhi* at *Khalal* stage purchased from Gurra Downs Date Company Pty Ltd, South Australia. The date was stored at -20˚C immediately after receiving and used within 3 months. The fruit calyx removed and washed gently to remove soil and field debris prior to processing followed by air-drying for about 15 min. Each individual fruit placed on a slow-continuous rotator (2 rpm) under light source (420 nm) at a distance of 10 cm. Based on the diameter of 23 mm of the date taken as an average of 10 fruits and the distance of 10 cm the light dosage at 10 min and 15 min was 180 J/cm^2 and 270 J/cm^2 respectively. It must be mentioned that this light dose was applied to the date surface while rotating at 2 rpm and not directly exposed for a 10 or 15-minute period. The fruit was sprayed by filtered CUR solution (3 - 4 µl) using 1000, 1400 and 1800 µM separately. There were three replicates for each treatment and each replicate had 4-5 dates. After spraying CUR, the fruit exposed immediately to visible light (420 nm) for 10 and 15 min. The different concentrations of CUR selected based on their efficacy in our previous study. The dates subjected to specific treatments combining the photosensitiser (PS) and light (L). Controls for this experiment included washed untreated date (L^-/PS^-), date inoculated with equivalent volumes of 30% propylene glycol only, and date inoculated with CUR solution but no light (L^-/PS^+) and date subjected to light but no CUR (L^+/PS^-). These controls allowed the determination of antifungal activity due to propylene glycol and allowed comparison of fungal levels found in fresh dates treated with neither CUR nor propylene glycol. After irradiation, the fruits were individually packed into sterile polyethylene bags and stored at two different temperatures (25˚ and 4˚C) observed daily for fungal growth and assessed according to the chart given in Table **1.** The given storage temperatures were selected to mimic the conditions of wholesale and retail markets.

2.3 Physicochemical changes of date after irradiation

The physico-chemical changes of date samples including moisture content, total sugar (TS) and total phenolic content (TPC) were determined by colorimetric assays as described by Al-Asmari et al., (2017b). The date pulp extraction performed using one gram of the treated date pulp homogenized with 20 ml ethanol 75% for 2 min then centrifuged at 4000 rpm for 10 min. The supernatant used to determine TS and TPC. For TS analysis, aliquots of 100 µl date ethanol extract was mixed in 100 µl phenol (80%) then 250 µl of sulfuric acid (95%) was added. The absorbance of the reaction measured in 96-well plate using a spectrophotometer at 490 nm (Tecan, Austria). For total phenolic content, aliquot of 25 µl ethanol extract was loaded to the 96-well and mixed with 125 µl Folin-Ciocalteu's phenol reagent (10%) freshly prepared. An aliquot of 125 µl sodium carbonate (7.5%, w/v) was pipetted to all the wells following incubation at 24°C for 30 min. The absorbance of mixture measured at 750 nm and the result expressed as Gallic acid equivalent (GAE/100g FW).

Statistical analyses

Statistical analyses performed using the XLSTAT-Pro software package version 7.0 (XLSTAT Addinsoft, Paris, France). Delete this line.

3. RESULTS AND DISCUSSIONS

3.1 The shelf life extension of date

The effect of a novel photosensitization-CUR treatment for date surface sanitation was investigated. Since fungal growth on fruit surface is an important index to confirm the quality and shelf life, any growth (slight, moderate or severe) that emerged on the date surface was considered as fungal infection (rotten) refer to Table 1, and treated as the end of shelf life. In

general, photosensitizing fresh date by CUR solution for 10 and 15 min increased shelf life significantly ($p<0.05$). The fruit photosensitized by 1400 µM CUR for 10 min, followed by storage at 4°C lasted more than three times (up to 98 day) clear from any fungal infection compared to controls that lasted only for 28 days under the same conditions. While the other CUR concentrations (*i.e.* 1000 and 1800 µM) exhibited slightly less effectiveness against infections. After 98 days of storage, only 3% among all photosensitized date was infected in comparison with 100% for control after 28 days. In other words, the shelf life of 97% of photosensitized fresh date stored at 4°C lasted up to 98 days free of fungal growth (Figure 1 and 2). In turn, the fresh date photosensitized by 1000, 1400 and 1800 µM CUR for 10 min, followed by storage at 30 °C showed two fold shelf life extension (up to 14 day) compared with control that lasted for 7 days. Interestingly, after 21 days of storage at 30 °C, only 6.6% of date photosensitized by 1800 µM CUR was infected and 13.3% for dates photosensitized by 1000 and 1400 µM, whereas the control was totally infected (100%) after 7 days of storage under the same conditions (Figure 1 and 3).

Although, there is no difference between 10 and 15 min of irradiation, the 1400 µM of CUR proved more efficient among all the given concentrations. **In a previous study by Al-Asmari et al (2017a) revealed that CUR concentration over 1000 µM showed no further fungal spore reduction due to the high turbidity of the mixture that may act as an optical screen scattering light instead of absorbing it. In the present 1400 µM of CUR was more effective as it was sprayed on the date fruit surface and was different to passing the light through a solution which is turbid.** In addition, date treated by CUR without light (PS^+/L^-) and light without CUR (PS^-/L^+) showed no differences when compared with control (data not shown). Clearly, the photosensitization-CUR possesses significant potency in sanitizing date surfaces resulting in extension of shelf life. A study carried out by Liu et al. (2016) on oyster using photosensitization-CUR at concentration of 10 µM and light

dose of 5.4 J/cm^2 revealed an extension of oyster's shelf life up to 12 days after treatment. Meanwhile, Temba et al. (2016) study exhibited a positive effect in minimizing *A. flavus* associated with maize kernels by reducing the growth. Additionally, no resistance to antimicrobial photosensitization has been reported to date, and it is thought very unlikely to occur (Brovko, 2010).

The most common way to distribute fresh dates is to harvest the fruits at the *Khalal* or *Rutab* stages, pack them in boxes and display them at the wholesale market, while dry dates may remain on the tree to dry (Al-Showiman et al., 1994). Obviously, as no processing on post-harvest fresh dates occurs, keeping dates in a chilled room is crucial to maintain their quality and prolong shelf life. However, fungi could remain growing slowly at low temperatures during the storage period, causing fruit deterioration. Therefore, sanitizing the skin of fresh dates before storage can eliminate or reduce microbes and extend the shelf life of fresh date fruits. Under aerobic conditions, the mold spores germinate and induce mycelial mass that are present on the skin of dates, with the possible secretion of mycotoxins. While spoilage yeasts may not be the main pioneers or initiators of fresh date spoilage, they ferment sugars in anaerobic conditions, producing acids that are associated with undesired aromas and flavors.

Despite photosensitization mediated by several PSs has been widely studied *in vivo* for medical purposes, implementing of successful experiments in the food processing are limited. This could be due to the challenge of finding suitable food grade photosensitizers to use in food processing. CUR is a natural compound and safe for human consumption. The European Union Food Safety Authority (EFSA) has agreed based on the available scientific data that CUR is not carcinogenic, but allocated an ADI of 3 mg/kg bw based on the no-observed-adverse-effect-level (NOAEL) of 25 – 320 mg/kg bw/day.. EFSA also noted that intake of CUR from the normal diet amounts to less than 7% of the 3 mg/kg bw ADI (EFSA, 2010, 2014). The action between CUR and light in presence of oxygen generates highly

cytotoxic singlet oxygen ($^1O_2$). This substance is known to suppress the growth of microorganisms. Although photosensitization has shown promise as a clean green technology and as an alternative to chemical fungicides, its applications for cleaning and disinfection are still in its infancy.

3.2 Physico-chemical characteristic changes of photosensitized date

As ready-to-eat food, any changes in physico-chemical characterization may influence the quality of date. Photosensitization-CUR, a cold sensitization technology targets the microorganisms on the surface of date. During treatment, the temperature of photosensitized date did not exceed one degree Celsius. In addition, the short wavelength (420 nm) utilized in the present study was unable to penetrate deeply into the fruit tissue. Although alterations in date characteristic were not visually observed, biochemical properties including total phenolic content, antioxidant activity and total sugar were determined. The result indicated that photosensitization-CUR processing had no adverse effect on the tested biochemical properties. No changes in moisture content, TS and TPC were observed (Table 2). Overall, photosensitization-CUR treatment showed a beneficial effect in sanitization of date surface, extending its shelf life and preventing quality deterioration. The low content of fat and protein in date could also prevent oxidization during irradiation.

4. CONCLUSION

Controlling fungal contamination becomes increasingly difficult because of the emergence of antifungal-resistant strains. One possible alternative and promising approach for sanitizing food surface is photosensitization-CUR treatment. The fresh date photosensitized by the combination of visible blue light and CUR and stored at 4 and 30°C showed significant increase in its shelf life. A 10 min irradiation in the presence of CUR at a concentration of 1400 µM extended the shelf life up to 14 days when stored at 30° and up to 98 days at 4°C

compared to 7 days and 28 days respectively for controls. Overall, the approach of photosensitization-CUR treatment has successfully demonstrated fungicidal activity against a broad range of pathogenic and food spoilage fungi, and extended the shelf life and safety of fresh dates intended for human consumption. This study is of great importance in developing an alternative novel fungicidal approach for different food commodities.

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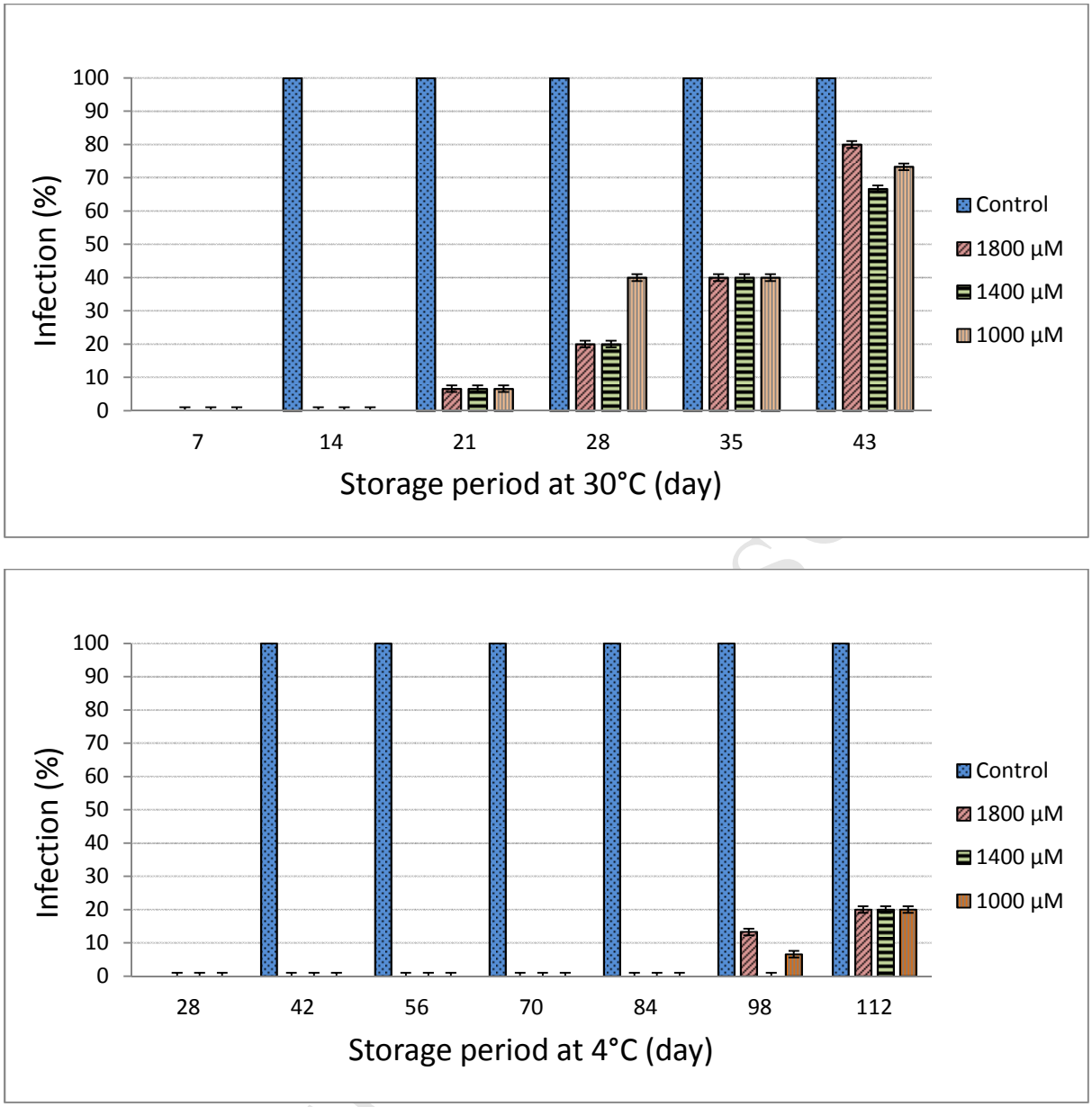


Figure 1. Effect of photosensitization-CUR at various concentrations on fungal (mycelial) growth occurring on fresh date surface stored at 4° C and 30° C.

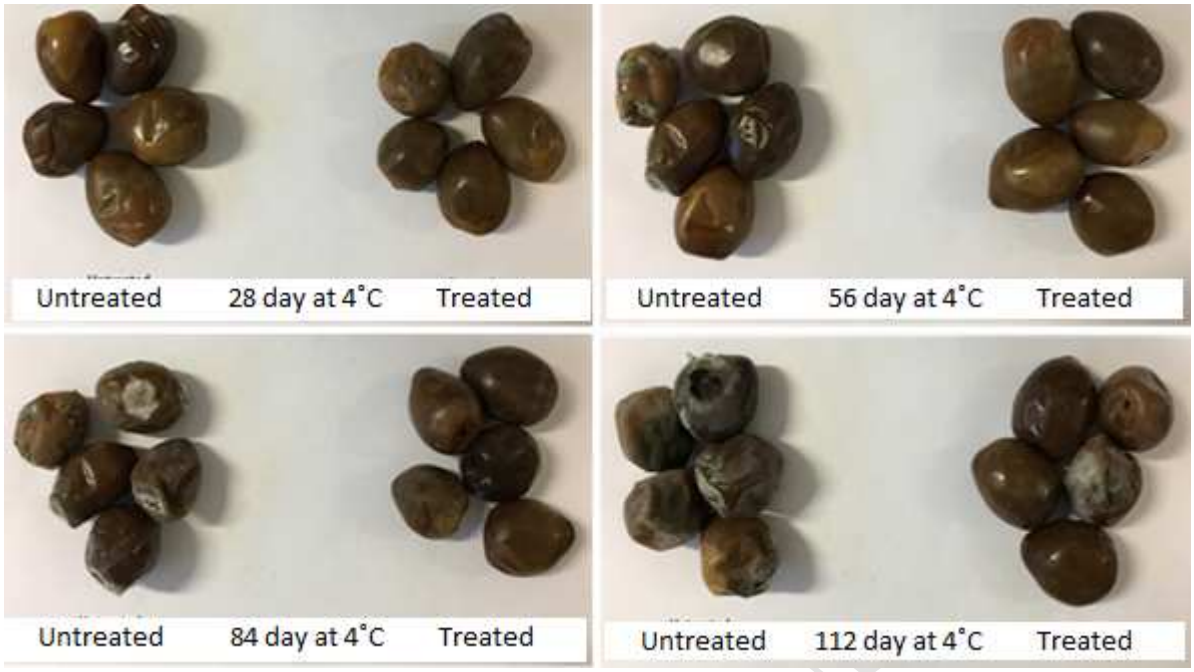


Figure 2. Effect of a photosensitization-CUR treatment using 1400 µM of CUR for 10 min irradiation on the shelf life of fresh dates stored at 4˚C. Fungal/mycelia growth was observed on control samples after 28 days while photosensitized dates lasted up to 84 days with no fungal growth under the same conditions

Figure 3. Effect of a photosensitization-CUR treatment using 1400 µM of CUR for 10 min irradiation on the shelf life of fresh dates stored at 30˚C. Fungal/mycelia growth was clearly observed on control samples after 7 days while photosensitized dates lasted up to 14 days with no fungal growth under the same conditions.

Table 1 - Images of infected/non infected dates by fungi showing the guidelines for rejection of date fruit

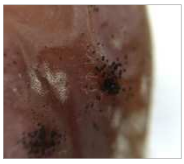
Degree of fungal infection	Date fruits			Consider as infected/non infected
No mycelia growth				Not infected ✓
Slight mycelia growth				Infected ✗
Moderate mycelia growth				Infected ✗
Severe mycelia growth				Infected ✗

Table 2. The changes of physio-chemical characteristics of fresh date prior and post photosensitization-CUR treatment

Physio-chemical characteristic	Untreated date	Treated date
Moisture content	54.63 (±0.40)	55.57 (±1.14)
Total sugar (g/100g FW)	1.19 (±0.03)	1.22(±0.03)
Total phenolic content (mg GAE/g FW)	27.83 (±0.34)	27.80 (±0.25)