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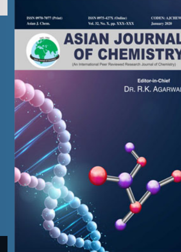

Effects of Development and Ripening Stage on Phytochemical Compositions, Antioxidant and Antibacterial Activities of Date Palm Fruits

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Edible parts of two dates cultivars (Deguel ahmar and Tanslit) at four maturation stages khellal, besser, rutab and tamr were analyzed for their phytochemical composition (total phenolic, total flavonoid and condensed tannins contents) as well as antioxidant and antibacterial activities. The antioxidant activity evaluated *in vitro* using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2′-azino-*bis*(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical and ferric reducing power. Antibacterial activity of extracts were evaluated *in vitro* by the agar disk diffusion method against five pathogenic bacterias trains: *Bacillus subtilis* ATCC 6633, *Enterococcus* ATCC 3315, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 7320. Results suggested that the content of these phytochemicals are very important at khellal stage and then decreased during maturation followed by a decrease in the antioxidant activity.

Keywords: Phytochemical composition, Antioxidant activity, Antibacterial activity, Date palm, Maturation stage.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is an important fruit tree in Arab countries due to its commercial and economic usefulness. Algeria is among leading countries in date production. Algerian oases contain override 900 cultivars and each region has its own qualities. These cultivars names differ from one region to another, thus we may find the same name reffers to different cultivars [1]. This fruit is the only available basic food for the inhabitants of desert and dry areas. It has an important antioxidant activity due to the water-soluble compounds with potent free radical-scavenging effects, such as flavonoids and its importance is not limited to food benefit, but used for cosmetics and pharmaceuticals. However, its special characteristics have increased the interest of industrial applications of this fruit [2,3]. The natural antioxidants compounds are now on a large scale used in cosmetic products and the application of these antioxidants could replace the application of synthetic antioxidants [4]. The date pass through four distinct stages of ripening termed in Arabic khellal for the immature green, besser for the mature full coloured, rutab the soft brown at most and tamr the hard raisin-like stages of development or they termed kimiri for the green then khellal, rutab and tamr as it is called in the Middle East countries.

Maturity stage is an important factor, which may affect the compositional quality of fruit and vegetables. During ripening, biochemical and structural are changing and these changes determine the fruit quality features [5]. Despite the importance of phonotypical and biochemical properties responsible for shape, taste and nutritional value for the industrial use of date and the responsibility of development stage for compositional quality of fruits [6,7], the publications in this context are limited.

Thus the aim of the present study was to determine the phytochemical compositions, antioxidant and antibacterial activities of two date palm cultivars grown in Ouargla region in Southeast of Algeria at four maturation stages. The results of this study can lead to identification of the optimal maturity stage for the harvest of date with high composition of polyphenols and good biological properties. Also it may be encouraging industrial applications of non-edible stages like pharmaceutical and cosmetics industries.

EXPERIMENTAL

The plant material selected in the present study was consisted of two cultivars of date palm (*Phoenix dactylifera* L.) namely, Deguel ahmar and Tanslit. For realization of this

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work, harvest was made during the different phenological stages from June to November.

Preparation of crude extracts: The date fruits were cleaned with tap water, the seeds were removed from date, edible part was cut to small pieces with a sharp knife and dried in dark at room temperature until we get a constant dry weight. The samples (50 g) of dried date at different maturity stages were extracted with 150 mL methanol-water (4:1, v/v) for 24 h and finally the extracts were filtered using Whatman No.1. The residue of each extract was centrifuged at 3500 × g for 20 min. The supernatant concentrated under reduced pressure at 40 ºC using a rotary evaporator until getting the constant weight. The methanol crude extract was kept in dark glass bottles at -40 ºC until used. The storage conditions were the same for all extracts.

Total phenolic content (TPC): Total phenolic content (TPC) of methanolic extracts was carried out by the colorimetric method [8]. A volume of 0.5 mL of gallic acid standard or dilution extracts were mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 3 mL of sodium carbonate solution (7.5 %, w/v). The reaction mixture incubated in dark at room temperature for 30 min. The absorbance was measured at 765 nm. The TPC was determined from standard gallic acid curve and expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW). All the measurements were taken in triplicate.

Total flavonoid content (TFC): Total flavonoid content of methanolic extracts was determined by the colorimetric method using aluminum chloride reagent and quercetin as standard [9]. A volume of 2 % $AlCl_3$ ethanol solution was added to volume of extract. The absorbance was determined at 430 nm. The calibration curve was prepared with quercetin and the results were expressed as milligrams of quercetin equivalents per 100 g of dry weight (mg QE/100g DW). All the measurements were taken in triplicate and the mean values were calculated.

Condensed tannins contents (CTC): Total tannin content was measured using Heimler method [10]. A 3 mL of 4 % ethanol vanillin solution and 1.5 mL of conc. HCl were added to 0.4 mL of extract. The mixture was allowed to stand for 15 min and the absorbance of pink colour was measured at 500 nm. Results were expressed as milligrams of catechin equivalents per 100 g of dry weight (mg CE/100 g DW). All the measurements were taken in triplicate.

DPPH radical scavenging assay: Free radical scavenging activity of methanol extract was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Different dilution of extracts were prepared from each extract. About 1 mL of 0.25 mmol/L DPPH solution in ethanol was added to 2.5 mL of sample solution and incubated for 30 min in dark at room temperature and the absorbance was recorded at 517 nm.

Each test was taken in triplicate and the mean values were calculated. The percent inhibition was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

In this work, antiradical efficiency (AE = $1/EC_{50}$) will be expressed.

ABTS assay: ABTS assay was measured as described by Re *et al*. [11]. The ABTS radical cation ($ABTS^{•+}$) was produced by the reaction of 7 Mm ABTS and potassium per sulfate (2.45 mM) in the dark at 23 ºC for 12-16 h. The $ABTS^{•+}$ solution was diluted with methanol (80 %) to an absorbance of 0.700 ± 0.002 at 734 nm.

Diluted $ABTS^{•+}$ solution (975 µL) was added to 25 µL of sample or methanol for blank or Trolox solution as standard and the absorbance was recorded at 734 nm. Antioxidant activity was expressed in terms of Trolox equivalents (µM/g).

Ferric reducing power assay: The reducing power of date extracts was estimated according to the reported method [12] with slight modifications. A 1 mL of ech extract was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1 %). The mixture obtained was incubated at 50 ºC for 20 min. Then, 2.5 mL of 10 % trichloroacetic acid were added. The solution obtained (2.5 mL) was mixed with 2.5 mL of deionized water followed by the addition of 0.5 mL of 0.1 % $FeCl_3$. The absorbance was measured at 700 nm. Reducing power was expressed (mM) as ascorbic acid equivalents antioxidant capacity (AEAC). All analyses were carried out in triplicate.

Antibacterial activity: Samples preparation and extraction of phenolic compounds from date palm was done as described earlier [9]. Dried extracts were dissolved in 100 % dimethyl sulfoxide.

Microorganisms: Five reference strains were chosen for antibacterial activity: Gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Enterococcus* ATCC 3315, *Staphylococcus aureus* ATCC 43300 and Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 7320.

Sensitivity test (agar-diffusion method): The antibacterial activity of the extracts was performed according to the disc diffusion method [13] with slate modification using Muller-Hinton agar. Agar after his thawed, it was poured in petri plates and then left to dry.

Cultures suspended in sterile saline (0.9 % NaCl) and the cell density was adjusted to 0.5 McFarland. Sterile 6 mm paper discs were filled with 10 µL of extracts (100 mg/mL). Before incubation, all petri-dishes were stored in the dark at 4 ºC then were incubated at 37 ºC for 24 h.

Antibacterial activity was determined by measuring the diameter of the inhibition zone in millimeters. Negative control (100 % DMSO) was used under the same conditions. Experiments were carried out in three replicates and the mean diameter of the inhibition zone was calculated.

RESULTS AND DISCUSSION

Total phenolic content (TPC): The total phenolic content during different maturity stages of Deguel ahmar and Tanslit are presented in Table-1. These results showed that the maturation has an effect on the TPC of date palm varieties where significant differences between the TPC of all samples. Maximum phenolic content was found at the khellal stage where Deguel ahmar had an significant amount (1596.25 ± 57.45mg GAE/100 g DW). TPC decreased significantly from khellal to tamr stage at the two cultivars selected. At tamr stage, Tanslit had the highest value (142.71 ± 0.43 mg GAE/100 g DW) and the

TABLE-1
TOTAL PHENOLIC, FLAVONOID AND CONDENSED TANNIN CONTENTS OF DEGUEL AHMAR AND TANSLIT DATE FRUITS DURING DIFFERENT STAGE OF RIPENING

Cultivars	Stage	TPC (mg GAE/100 g DW)	TFC (mg QE/100 g DW)	CTC (mg CE/ 100 g DW)
Deguel ahmar	Khellal	1596.25 ± 57.45	7.30 ± 0.08	598.79 ± 14.02
	Besser	647.03 ± 20.68	3.81 ± 0.09	249.42 ± 17.86
	Rutab	93.83 ± 2.24	1.94 ± 0.06	4.28 ± 0.12
	Tamr	86.54 ± 1.40	2.32 ± 0.05	9.16 ± 0.13
Tanslit	Khellal	496.98 ± 15.20	5.65 ± 0.06	268.29 ± 3.91
	Besser	402.00 ± 3.27	4.25 ± 0.07	167.43 ± 5.87
	Rutab	152.20 ± 2.06	4.60 ± 0.15	38.09 ± 0.49
	Tamr	142.71 ± 0.43	2.67 ± 0.07	16.67 ± 0.22

least value was found in Deguel (86.54 ± 1.40mg GAE/100 g DW). These values of total phenols are close to those reported for some Saudi Arabia dates [14]. The decreases of TPC in date fruit was reported in other study of Shahdadi *et al*. [14]. This decrease in total phenolic level may be due to the oxidation of phenolic content by polyphenol oxidase or the contribution of phenolic to the biosynthesis of flavylium ring of anthocyanin [15,16]. The decrease in TPC during maturation was also reported in other fruits jujube, blackberry and blueberry [17-19].

On the contrary, TPC contents increased dramatically with harvest time in Jordanian dates [20]. These differences may be due to cultivar variation and genetic variability among palms.

Total flavonoid content (TFC): The results obtained from TFC (Table-1) show a decrease of total flavonoid during the ripening of date fruits. The highest level of TFC was detected at khellal stage with concentration 7.30 ± 0.08 and 5.65 ± 0.06 mg GAE/100 g DW, respectively for Deguel ahmar and Tanslit. For Deguel ahmar, there was no significant difference between rutab and tamr stage. For Tanslit, total flavonoid reaches its lowest value at tamr stage (2.67 ± 0.07mg CE/100g DW). This decrease indicates that drying process may have a destructive effect on flavonoid. The present results are similar to sing's findings especially khasab and khalas cultivars at the tamr stage, they are different to those reported by Odeh *et al*. [20], which found that flavonoids content increased with the stages of maturity in some Jordanian dates.

Condensed tannins content (CTC): Natural antioxidants such as condensed tannins are more attractive to researchers for medical product or food usage besides antioxidant activities. Strong antityrosinase activities of condensed tannins has been reported in several studies [22].

Comparing the four maturation stages in Table-1, it was found that khellal stage was characterized by the greatest level of CTC (598.79 ± 14.02 mgCE/100 DW) and (268.29 ± 3.91 mgCE/100 DW), respectively for Deguel ahmar and Tanslit. This result confirms the astringent taste of this stage. According to the present results, the rutab stage of Deguel ahmar showed the lowest CTC of 4.28 ± 0.12 mg CE/100DW then increased at the tamr stage (9.16 ± 0.13 mg CE/100DW). Similar findings from the rutab stage to tamr was also reported by Myhara *et al*. [23]. In contrast, CTC decreased in Tanslit even recorded the lowest value at tamr stage (16.67 ± 0.22 mg CE/100 DW) and this result is an agreement with the results found by Awad *et al*. [15]. The differences between our cultivars should be attributed to the genetic variability and to the nature of tannin contents [5].

DPPH radical scavenging activity: The results of DPPH radical scavenging activity of extracts are shown in Fig. 1. For two cultivars, the most active was estimated in date fruits at the khellal followed by besser, rutab and tamr stage. A strong correlation was found between AE values and total phenolic contents ($R^2 = 0.85$) in Deguel and ($R^2 = 0.83$) in Tanslit. Also, DPPH radical scavenging activity was highly correlated with condensed tannins content ($R^2 = 0.85$) and ($R^2 = 0.91$), respectively for Deguel and Tanslit. A highly positive relationship was found between total phenolic and tannins of date cultivars in other reported studies [5,14]. In contrast, Chaira *et al*. [24] and Al-Asmari *et al*. [25] found the negative correlation. This is due to the difference of phenolic compound from a cultivar to another.

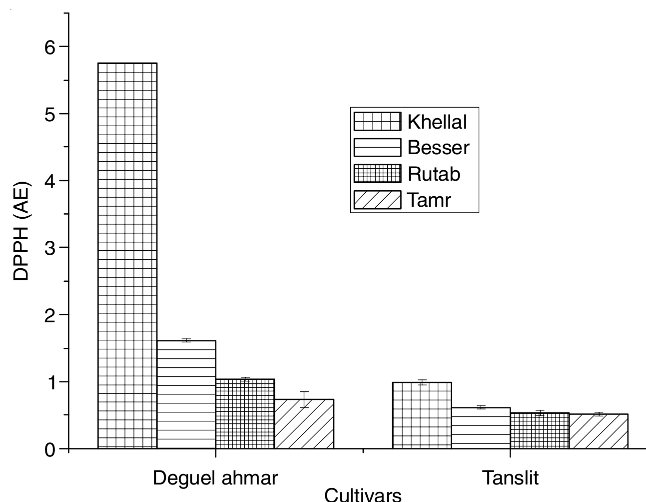

Fig. 1. Changes in antiradical efficiency = $1/IC_{50}$ (DPPH) of date palm cultivars during development and ripening

ABTS assay: The antioxidant activity determined by ABTS in two date cultivars at the four stages are shown in Fig. 2. With regard to Dguel ahmar results obtained showed high antioxidant activity at besser stage (151.73 ± 7.51 µmol TE/g) and the lowest value was recorded at rutab stage (16.53 ± 0.91 µmol TE/g). This result is different from DPPH assay, which can be attributed that components of the stages reacted differently with chemicals involved in different antioxidant testes. The khellal stage has a high activity at Tanslit with a value of 71.07 ±0.6 µmol TE/g then it increases.

Reducing power assay: The results of reducing power of two date palm cultivars as a function of ripening stage are shown in Fig. 3. A high difference from khellal to tamr stage is observed. The khellal is the potent reductant where Deguel ahmar recorded the highest values (208.36 ± 10.44mM) followed by besser stage (78.64 ± 3.96 mM).

TABLE-2
ANTIBACTERIAL ACTIVITY OF DATE CULTIVARS EXTRACTS AT VARIOUS RIPENING STAGES AGAINST PATHOGENIC BACTERIA (DIAMETERS OF GROWTH INHIBITION ZONES) USING AGAR DISC DIFFUSION

Cultivars	MS	*Bacillus subtilis* ATCC 6633	*Staphylococcus aureus* ATCC 43300	*Enterococcus* ATCC 3315	*Pseudomonas aeruginosa* ATCC 7320	*Escherichia coli* ATCC 25922
Deguel ahmar	Khellal	Not detected	14.10 ± 1.49	Not detected	Not detected	8.84 0.71
	Besser	Not detected	11.72 ± 1.13	7.21 ± 0.91	Not detected	7.69 ± 0.85
	Routab	9.98 ± 0.81	14.25 ± 1.62	9.48 ± 1.13	Not detected	10.01 ± 1.21
	Tamr	8.51 ± 0.93	18.81 ± 2.50	7.63 ± 1.05	Not detected	7.59 ± 1.82
Tanslit	Khellal	10.56 ± 1.36	15.72 ± 1.68	Not detected	Not detected	9.93 ± 0.57
	Besser	Not detected	9.67 ± 0.71	8.75 ± 1.15	Not detected	Not detected
	Routab	Not detected	15.94 ± 2.25	7.43 ± 0.69	Not detected	8.25 ± 0.26
	Tamr	Not detected	14.82 ± 1.95	Not detected	Not detected	Not detected

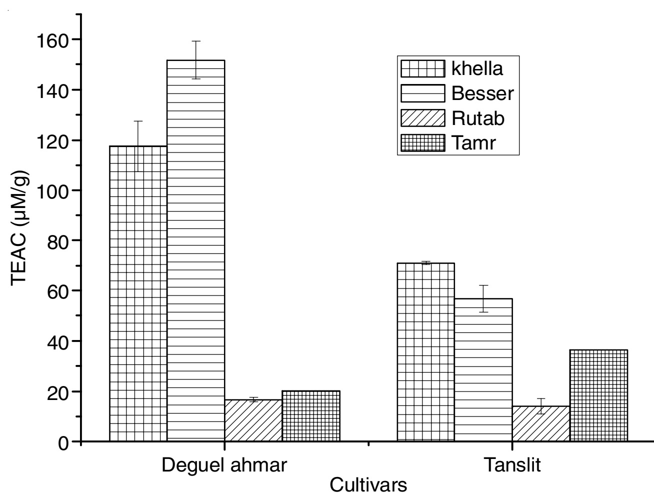

Fig. 2. Changes in radical scavenging activity (ABTS$^{•+}$) of date palm cultivars during development and ripening

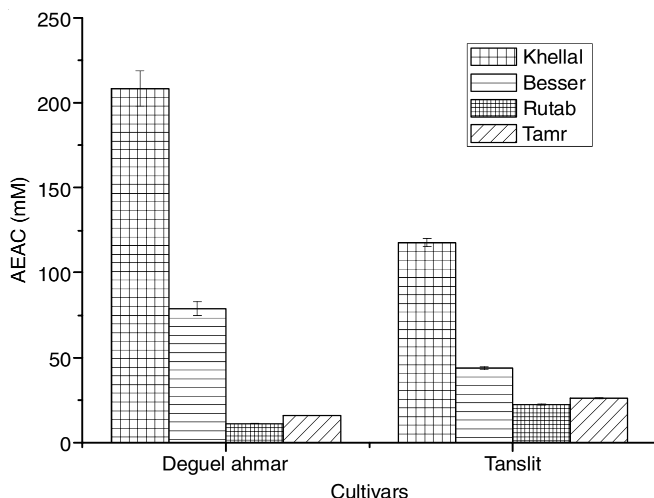

Fig. 3. Changes in reducing power (RP) of date palm cultivars during development and ripening

Regarding to maturation of dates from rutab to tamr, a marginally increased was observed in this two cultivars where Tanslit recorded the highest values at rutab (26.40 ± 0.41 mM) stage and tamr stage (22.40 ± 0.28 mM). Preent results are in good agreement with those found by Singh *et al*. [21], where a slight increase or non-existent from rutab to tamr stage in three cultivars from Sultanate of Oman is noticed.

Antibacterial activity: The results of antibacterial activity (Table-2) showed different degrees of growth inhibition, depending on the cultivar and maturation stage. *Staphylococcus aureus* was the most sensitive organism to the all extracts during all ripening stages (inhibition zone was ranged from 9.67 ± 0.71 to 18.81 ± 2.50 mm). In case of *Bacillus subtilis*, the most interesting activity was obtained from Tanslit at khellal stage with inhibition zone of 10.56 ± 1.36 mm, this may be due to the presence of rich active compounds at this stage. In the case of *Enterococcus*, results from agar diffusion method indicate that Deguel ahmar at routab stage showed the highest activity (10.01 ± 1.21 mM) as compared to other extracts.

No inhibitory effect on the tested bacteria was observed for *Pseudomonas aeruginosa*, while six date extracts were found to be active against *Escherichia coli* with diameter of inhibition zones ranging from 7.59 ± 1.82 to 10.01± 1.21 mm. Antibacterial activity of date palm fruit at different ripening stages studied by Saleh and Otaibi [26] but no study at khellal stage was conducted.

Date fruit exhibited good inhibitory activity against bacterial food pathogens. This is due to the presence of active phenolic compounds such as tannins and flavonoids [3] whose nature and amount play an important role in inhibition of bacteria [27].

Conclusion

The results obtained in the present study showed that the date palm fruit extracts demonstrated high phytochemicals composition and antioxidant activity during ripening from khellal to tamr stage. In that sense, from the nutritional point of view, to choose the date palm fruit at immature stage instead of tamr, late besser or rutab stages. On the other hand, it is also proposed to use date palm fruit at the khellal stage in pharmaceuticals and cosmetics products due to its richness with safe and natural antioxidants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Simozrag, A. Chala, A. Djerouni and M.E. Bentchikou, *Gayana Bot.*, **73**, 42 (2016).
2. H. Boudries, P. Kefalas and D. Hornero-Méndez, *Food Chem.*, **101**, 1372 (2007); https://doi.org/10.1016/j.foodchem.2006.03.043.

3. A. El Arem, E.B. Saafi, G. Flamini, M. Issaoui, A. Ferchichi, M. Hammami, A.N. Helall and L. Achour, *Int. J. Food Sci. Technol.*, **47**, 549 (2012); https://doi.org/10.1111/j.1365-2621.2011.02876.x.
4. I. Kusumawati and G. Indrayanto, ed.: Atta-ur-Rahman, Natural Antioxidants, In: Studies in Natural Products Chemistry, Elsevier, vol. 40, pp. 485-505 (2013).
5. E.A. Amira, S.E. Behija, M. Beligh, L. Lamia, I. Manel, H. Mohamed and A. Lotfi, *J. Agric. Food. Chem.*, **60**, 10896 (2012); https://doi.org/10.1021/jf302602v.
6. S. Ghnimi, S. Umer, A. Karim and A. Kamal-Eldin, *NFS J.*, **6**, 1 (2017); https://doi.org/10.1016/j.nfs.2016.12.001.
7. M.S. Haider, I.A. Khan, M.J. Jaskani, S.A. Naqvi, S. Mateen, U. Shahzad and H. Abbas, *Pak. J. Bot.*, **50**, 1069 (2018).
8. M. Al-Owaisi, N. Al-Hadiwi and S.A. Khan, *Asian Pac. J. Trop. Biomed.*, **4**, 964 (2014); https://doi.org/10.12980/APJTB.4.201414B295.
9. M. Djeridane Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal, *Food Chem.*, **97**, 654 (2006); https://doi.org/10.1016/j.foodchem.2005.04.028.
10. D. Heimler, P. Vignolini, M.G. Dini, F.F. Vincieri and A. Romani, *Food Chem.*, **99**, 464 (2006); https://doi.org/10.1016/j.foodchem.2005.07.057.
11. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med.*, **26**, 1231 (1999); https://doi.org/10.1016/S0891-5849(98)00315-3.
12. A. Kumaran and R.J. Karunakaran, *LWT-Food Sci. Technol.*, **40**, 344 (2007); https://doi.org/10.1016/j.lwt.2005.09.011.
13. S. Krimat, T. Dob, M. Toumi, A. Kesouri and A. Noasri, *J. Mater. Environ. Sci.*, **6**, 70 (2015).
14. F. Shahdadi, H. Mirzaei and A.D. Garmakhany, *J. Food Sci. Technol.*, **52**, 1814 (2015); https://doi.org/10.1007/s13197-013-1177-6.
15. M.A. Awad, A.D. Al-Qurashi and S.A. Mohamed, *Sci. Hortic.*, **129**, 688 (2011); https://doi.org/10.1016/j.scienta.2011.05.019.
16. O.A. Fawole and U.L. Opara, *Sci. Hortic.*, **150**, 37 (2013); https://doi.org/10.1016/j.scienta.2012.10.026.
17. S.Y. Wang and H.-S. Lin, *J. Agric. Food Chem.*, **48**, 140 (2000); https://doi.org/10.1021/jf9908345.
18. A.D.R. Castrejón, I. Eichholz, S. Rohn, L.W. Kroh and S. Huyskens-Keil, *Food Chem.*, **109**, 564 (2008); https://doi.org/10.1016/j.foodchem.2008.01.007.
19. B. Wang, C. Venkitasamy, F. Zhang, L. Zhao, R. Khir and Z. Pan, *LWT-Food Sci. Technol.*, **69**, 458 (2016); https://doi.org/10.1016/j.lwt.2016.01.077.
20. I. Odeh, F. Al-Rimawi, J. Abbadi, L. Obeyat, M. Qabbajeh and A. Hroub, *J. Food Nutr. Res.*, **2**, 499 (2014); https://doi.org/10.12691/jfnr-2-8-11.
21. V. Singh, N. Guizani, M.M. Essa, F. Hakkim and M. Rahman, *Int. Food Res. J.*, **19**, 1063 (2012).
22. H.-L. Feng, L. Tian, W.-M. Chai, X.-X. Chen, Y. Shi, Y.-S. Gao, C.-L. Yan and Q.-X. Chen, *Appl. Biochem. Biotechnol.*, **173**, 179 (2014); https://doi.org/10.1007/s12010-014-0828-z.
23. R.M. Myhara, A. AlAlawi, J. Karkalas and M.S. Taylor, *J. Sci. Food Agric.*, **80**, 2181 (2000); https://doi.org/10.1002/1097-0010(200012)80:15<2181::AID-JSFA765>3.0.CO;2-C.
24. N. Chaira, M.I. Smaali, M. Martinez-Tomé, A. Mrabet, M.A. Murcia and A. Ferchichi, *Int. J. Food Sci. Nutr.*, **60(Suppl. 7)**, 316 (2009); https://doi.org/10.1080/09637480903124333.
25. F. Al-Asmari, N. Nirmal, M. Chaliha, D. Williams, R. Mereddy, K. Shelat and Y. Sultanbawa, *Food Chem.*, **221**, 644 (2017); https://doi.org/10.1016/j.foodchem.2016.11.125.
26. F. Saleh and M. Otaibi, *J. Food Process. Technol.*, **4**, 12 (2013); https://doi.org/10.4172/2157-7110.1000285.
27. W. Kchaou, F. Abbès, R.B. Mansour, C. Blecker, H. Attia and S. Besbes, *Food Chem.*, **194**, 1048 (2016); https://doi.org/10.1016/j.foodchem.2015.08.120.