CONTROLLING THE ENZYMATIC BROWNING OF ALGERIAN DEGLET NOUR FRUIT

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Abstract. *Enzymatic browning in vegetal organs such as fruits and vegetables can produce undesirable color changes and unfavorably affect the taste, flavor, texture, and also the nutritional value. Dates' color is known to play a crucial role in determining the quality and value of the fruit. The color changes from the natural accepted golden color to unfavorable dark brown color during storage period.We investigated in this study, the natural anti-browning effects of the extracts, of different parts of Phoenix dactylifera L., on polyphenol oxidase and peroxidase enzymatic activity of the fruits of date palm, Deglet Nour variety, which are widely consumable by Algerian population due to its respective health benefits.The total phenolic and flavonoid content of hydro-methanolic extracts from pedicel, perianth and leaves of date palm were also evaluated. The results showed the leaves extract is a promising source of phenolic compound with value about 27.72±0,0005 mg of gallic acid equivalents (GAE) per g of dry weight (DW). Furthermore, higher flavonoids content 12.52±0.0355 mg rutin equivalent (RE) per g DW.The anti-browning activity analyzed using a UV-visible spectrophotometer indicated that all extracts showed no effect inhibition on enzymatic activities of polyphenol oxidase (PPO) and peroxidase (POD) from fruits of date palm. This study shows that different parts of date palm, Deglet Nour variety, cannot be used as effective, natural, non-toxic anti-browning agents.*

Keywords*: Enzymatic browning, Deglet Nour, anti-browning agents, phenolic compound, polyphenol oxidase and peroxidase.*

INTRODUCTION

Date palm tree belongs to *Arecaceae* family (Angiosperms, monocotyledon) consisting of about 200 genera and more than 2,500 species (Al-Alawi and al., 2017). The genus *Phoenix* includes 14 species, including *Phoenix dactylifera* L., distributed in the tropical or subtropical regions of the old World (Rekis and al., 2020).

The date palm tree is considered a symbol of life in the arid and semi-arid regions of the hot sphere, due to its tolerance to high temperatures, droughts, and salinity, compared to other fruit crop species (Rekis and al., 2020; Harkat and al., 2022).

Phoenix dactylifera L. is one of the oldest and main staple in Southwest Asia and North Africa (Rekis and al., 2020). It is mainly cultivated in Australia, Mexico, South America, southern Africa, and the United States of America, especially in southern California, Arizona and Texas (Al-Alawi and al., 2017).

Besides, the annual global production of 9.24 million tons (production increased considerably in the last 30 years). Algeria is considered as one of largest producers of dates in the world; with an average production of approximately 1.13 million tons/year. The variety

inventory has identified more than 940 cultivars and more than 13 million date palm trees (Harkat and al., 2022).

Date palm fruit is a significant source of nutrients and bioactive compounds such as carbohydrates, dietary fibers, minerals and vitamins with advantageous health benefits such as anticancer, antioxidant, antimicrobial, antidiarrheal and anti-inflammatory activities, among others (El Bakouri and al., 2021). In addition, the main phenolic classes and member phenolic compounds which identified in date fruit are gallic, protocatechuic, *p*-coumaric acid, and ferulic acid, as well as some cinnamic acid derivatives (Ghnimi and al., 2017; Sarraf and al., 2021). Depending on the maturity and ripeness stages during growth and development of the dates, different external and internal changes are observed such as color, sweetness, texture and chemical composition (Al-Alawi and al., 2017).

Marketability and economical value of dates in local and international markets, and its related products are determined by many criteria such as color, flavor, taste (sugar level), moisture (26–30%) and absence of defects such as insect, cracks and surface damage. The deterioration of dates during storage is a major concern and results in changes in appearance and taste (Al-Alawi and al., 2017; Al-Amrani and al., 2020).

Browning reaction is the process by which there is a color change to brown or dark brown over time, seen in food during pre-preparation, processing and/or storage. In general, it is a major chemical and physiological loss that affects fruits' quality and taste (Moon and al., 2020; Chandrasekhar, 2021). This reaction is one of the most serious phenomenon that take place in dates during storage and affect color (Al-Amrani and al., 2020), and it can either be non-enzymatic or enzymatic browning, depending on the involved mechanism (Moon and al., 2020; Chandrasekhar, 2021).

In food products, the non-enzymatic browning reaction generates a brown-colored substance through a chemical reaction involving a single compound or multiple constituents in food, without having any enzyme involved. This reaction includes the Maillard reaction, caramelization, and ascorbic acid oxidation (Moon and al., 2020).

However, the enzymatic browning reaction is a process that involves enzymatic action and considered as an indicator of quality loss responsible for many fresh and processed fruits and vegetables such as dates, banana, apricot and potato (Al-Amrani and al., 2020; Liu and al., 2021).

Enzymatic browning mostly occurs in fruit and vegetable products during harvesting, transportation, storage, and processing; subsequently, it influences the sensory and nutritional values of food products (Moon and al., 2020).

The primary reason for browning may be the disruption of the membrane system. Once fruits and vegetables are damaged mechanically or in an adverse environment, phenolic compounds inside them will be oxidized to quinones and ends with melanin-like transformation by polyphenol oxidase (PPO) or peroxidase (POD), when oxygen is available. Additionally, lipoxygenase (LOX) activity and accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were exposed to participate in browning development. Similarly, phenol and phenylalanine ammonia lyase (PAL) play a key role in the occurrence of browning (Qiao and al., 2021; Kaewjumpol and al., 2021).

There are several approaches for the prevention of enzymatic browning such as addition of some chemical substances such as organic acids, antioxidants, chelating agents, heat treatment, cold storage, radiation, and other advanced techniques like radio frequency heating (Al-Amrani and al., 2020; Moon and al., 2020; Kaewjumpol and al., 2021).

However, some methods are limited to use, due to the high cost, low operability or potential hazard concerns. Focusing on a natural, safe and low-cost anti-browning substance is of interest (Kaewjumpol and al., 2021).

Several anti-browning extracts have been demonstrated to efficiently reduce browning in fresh cuts (Kaewjumpol and al., 2021). Therefore, there are very limited reports that talk on enzymatic oxidation of phenolic compounds in dates during ripening and storage (Al-Amrani and al., 2020).

Because of the importance of enzymatic browning and the loss of quality of the dates in the Tamer stage during ambient storage, the objective of the present work was to suggest a practical treatment or preservation method to stop or slow down the browning enzymes (PPO and POD) to preserve the surface color of dates.

Treatment with organic acid or sulfur dioxide has shown good results in other fruits; therefore, few studies have been conducted to inhibit browning by natural by-products. It is worthwhile to mention that to the best of our knowledge, practically, no studies have been investigated on the inhibition of enzymatic browning on date fruits by natural by-products.

The main objective of this study is to investigate the natural anti-browning effects of the extracts of different parts of *Phoenix dactylifera* L., on polyphenol oxidase and peroxidase enzymatic activity in Deglet Nour variety.

MATERIALS AND METHODS

1. Sample Collection.

For this study, Deglet Nour dates (the queen of all dates) of 2015 production were used at Tamer stage. The dates were received from a local farm in Metlili, Ghardaïa (situated at 600 km to the south of the capital of Algeria (Algiers), GPS: North 32°21', Wes 3°31', and Altitude: 542 m). The samples were selected identified in terms of size, color, ripening stage, without damaged and stored in a freezer at -20°C until further analysis.

The perianth, pedicel and leaves of date palm, of the same cultivars, were selected for testing as browning inhibitors. They were air-dried under the shade at room temperature and grinded to convert the seeds to powder.

2. Preparation of inhibitor extracts

The organic extracts were prepared by the method described by Djeridane and al., (2006), with slight modifications. The powder of by-products of date palm is macerated in hydro-methanolic solution of 80% (v/v) with a ratio 1/20 (w/v), for 48 h at room temperature. The extracts were filtered and then evaporated. The residue obtained was recovered in a certain volume of methanol.

3. Determination of Total Phenolic Content (TPC)

TPC of inhibitor extracts was identified using the Folin-Ciocalteu method (Singleton and Rossi, 1965). The extract (100 µl) was mixed with 0.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) for 5 min at room temperature. A volume of 2 ml of aqueous Na_2CO_3 (2%) was added and the mixture was vortexed and incubated in the dark at room temperature. After 30 min, the absorbance was measured at 760 nm by a UV-visible spectrophotometer (SPECTROSCAN 40). The TPC was calculated from standard gallic acid curve and expressed as milligrams of gallic acid equivalents per 100 g of dry weight of by-products of date palm for three replicates (mg GAE/100 g DW).

4. Determination of Total Flavonoid Content (TFC)

TFC of the inhibitor extracts was measured according to the colorimetric assay (Djeridane and al., 2007). A volume of 1 ml of the extract was added to 1ml of $AlCl_3$ (2%). Test tubes were incubated at room temperature for 20 min. The absorbance of the mixture was

determined at 409 nm. The TFC was determined from standard rutin curve and expressed milligrams of rutin equivalents per 100 g of dry weight of by-products of date palm for three replicates (mg QE/100 g DW).

5. Enzyme Crude Extract

A crude extract of the PPO and POD enzymes was obtained using the method described by Al-Jassabi and al. (2013), with slight modification. In this method, 30 g of date sample was cut quickly into thin slices and homogenized in 100 ml of 100 mM sodium phosphate buffer (pH 6.8) containing 0.5% (w/v) polyvinylpyrrolidone for 60 min in an ice bath. The homogenate was then centrifuged at 4,000 rpm for 60 min at 4°C. The supernatant was collected and filtered. The filtered supernatant was used immediately for PPO and POD activity assessment.

6. PPO Activity Assessment.

The method described by Lee and al. (2007) was used in this study with minor modifications. The enzyme crude extract was incubated in water bath for 5 min at 30°C before the reaction to optimize and standardize enzyme activity. After the incubation, 0.5 ml of 0.2 M of the pyrocatechol (substrate) solution was added to 0.95 ml of 0.1 M sodium phosphate buffer (pH 6.8). Then, 0.05 ml of the crude enzyme extract was added. Immediately, the measurement was taken at 420 nm every 30 s for 5 min. Enzyme activity was calculated from the steady line at the early stage of the reaction. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

7. POD Activity Assessment.

POD activity was assayed using the method described by Ponce and al. (2004) with slight modification. The enzyme extract was incubated in water bath for 5 min at 30°C before the reaction. The POD reaction mixture is composed of 2.87 ml of the substrate solution (containing 10 ml of guaiacol (1 %), 10 ml of hydrogen peroxide (0.3 %) and 100 ml of sodium phosphate buffer (0.1 M, pH 6.5)), 0.03 ml of sodium phosphate buffer and 0.1 ml of crude extracts. After rapid mixing, the absorbance at 470 nm was recorded every 30 s for 5 min. Enzyme activity was calculated from the steady line at the early stage of the reaction. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in absorbance per minute.

8. Analysis of Inhibitory Activity

The potential of the by-products of date palm as inhibitors of extracted PPO and POD from dates of Deglet Nour was investigated using pyrocatechol and guaiacol as substrates, respectively. Ascorbic acid (0.2 M) was used as commercial inhibitors of PPO and POD.

Briefly, the reaction mixture for inhibition the PPO activity included 50 µl of the enzyme crude extract, 0.5 ml of inhibitor extracts, 0.45 ml of phosphate buffer (0.1 M, pH 6.8) and 0.5 ml of 0.2 M the pyrocatechol (substrate) solution. The absorbance value was recorded at 420 nm every 30 s within 5 min.

The POD reaction mixture on presence of inhibitor is composed of 2.87 ml of the substrate solution (composed of 10 ml of guaiacol (1 %), 10 ml of hydrogen peroxide (0.3 %) and 100 ml of sodium phosphate buffer (0.1 M, pH 6.5)), 0.03 ml of inhibitor extracts and 0.1 ml of crude extracts. After rapid mixing, the absorbance at 470 nm was recorded every 30 s for 5 min.

The inhibition percentage of dates PPO and POD activities was calculated using the following equation:

$$I\ (\%) = ((A_c - A_i)/A_c) \times 100$$

Where A_c is the absorbance in the control and A_i is the absorbance in the treatment.

RESULTS AND DISCUSSIONS

1. Determination of TPC and TFC

Yield, total phenolic and flavonoid contents of by-products extracts for Deglet Nour variety using hydro-methanolic (80%) are shown in Table 1.

Table 1.

Total phenolic content (TPC), total flavonoid content (TFC) and extraction yield of different extracts of date palm.

Sample	Yield %	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg RE/g DW)
Pedicel	21.53%	12.74±0.0180	02.57±0.0220
Perianth	32.69%	11.90±0.0280	03.23±0.0085
Leaves	17.86%	27.72±0.0005	12.52±0.0355

It is evident that the perianth extract have a higher yield as compared to the pedicel and leaves extracts. While the lowest yield (17.86%) was obtained from leaves extracts. The extraction of phenolic compounds yield was significantly affected by organ, variety, chemical nature of plant, extraction solvents and sample particle size (Messaoudi and al., 2021).

Total phenolic content of different extracts of date palm was estimated by Folin-Ciocalteu reagent (FCR), the results are expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW). Total phenolic content of the different extracts were ranging from 11.90±0.0280 and 27.72±0.0005 mg/g GAE/g DW. The leaves extract exhibited the highest total phenolic content (27.72 mg/g) (Figure 1).

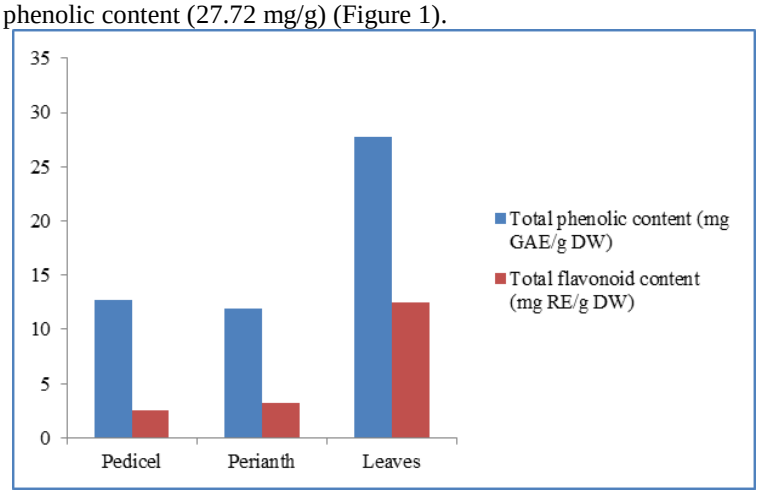

Fig. 1. Total phenolic content (TPC) and total flavonoid content (TFC) of different extracts of date palm.

Total flavonoid content was determined using spectrophotometric method with aluminum chloride. The content of flavonoid expressed as rutin equivalents per gram dry weight (mg RE/g DW), varied from 02.57±0.0220 to 12.52±0.0355 mg RE/g DW. The highest amount of flavonoid contents was determined for leaves extract, followed by perianth extract and pedicel extract.

The variation in TPC and TFC may be owing to the chosen variety of dates, the total variation in growing conditions, wider genetic variation and the methods of extraction (Zineb and al., 2012).

2. Kinetic characteristics of PPO and POD from Deglet Nour dates

The dependence of PPO and POD velocity on substrate concentration was studied. Consequently, the kinetics parameters of purified PPO and POD from Deglet Nour dates were calculated using pyrocatechol and guaiacol as substrates respectively at optimal conditions.

Substrate saturation curves for pyrocatechol and guaiacol indicated that PPO and POD follows simple Michaelis-Menten kinetics. Kinetic parameters values (Vmax, Km) of PPO and POD from Deglet Nour dates are shown in Table 2.

Table 2

Kinetic parameters of PPO and POD from Deglet Nour dates.

	Vmax (UE)	Km (M)	Vmax/Km (UE/M)
PPO	19.20	0.22	87.27
POD	18.28	0.03	609.33

Km value reflects the affinity of enzyme towards its substrate, smaller values shows greater affinity for its substrate. However, the main principle for choosing the best substrate is by the highest catalytic efficiency (Vmax/Km). Also, the catalytic efficiency seems to be dependent on the substrate used as well as the source of the enzyme (Benaceur and al., 2020).

The POD seems to have a great affinity for guaiacol (0.03 M). In contrast, the lower catalytic efficiency for pyrocatechol was also found for PPO of Deglet Nour dates.

Although, another work on Deglet Nour and Tadela dates showed that the values of Km was found to be lower (37.7 mM and 35 mM for pyrocatechol as substrate respectively) compared with our results (Benaceur and al., 2019; Benaceur and al., 2020).

The values of kinetic parameters vary according to many factors such as the substrate used, the source of enzyme, extraction and purification methods, which could affect the kinetic behavior of PPO and POD (Benaceur and al., 2019).

3. Inhibitory effect of the selected extracts on browning enzymes activities

The potential of the extracts in reducing or blocking the enzymes activity responsible for browning *in vitro*, PPO and POD, are of high interest. The effectiveness of extracts of *Phoenix dactylifera* in restraining these enzymes' activity was examined using the different parts of date palm, Deglet Nour variety. Table 3 shows the inhibitory effect of perianths, pedicels and leaves extracts on PPO and POD activities of fruits of date palm with pyrocatechol and guaiacol as substrate.

Table 3

Effect of extracts of *Phoenix dactylifera* as naturals inhibitors on PPO and POD activities of fruits of date palm, Deglet Nour variety.

Sample	The inhibition percentage of dates PPO (%)	The inhibition percentage of dates POD (%)
Pedicel	-	-
Perianth	-	-
Leaves	-	-
Ascorbic acid	32.32	79.46

In general, the different extracts of *P. dactylifera* might not be good inhibitors for PPO and POD activities of fruits the Deglet Nour as no inhibition percentage was obtained with a slight increase in enzymatic activity was noticed.

In contrast, the ascorbic acid provided the greatest inhibition of PPO and POD activities at concentration of 0.2 M as follow: 32.32 % and 79.46 % respectively. Ascorbic acid is the most widely used inhibitor agents. In fact, it is acted as an antioxidant reducing *o*-quinone back to the original phenol compound (Baltas and al., 2017).

PPO is considered to be the critical enzyme involved in enzymatic browning, which catalyzes phenol oxidation, quinine polymerization and dark pigment generation. Meanwhile, POD is found to participate in browning reactions via H_2O_2 during the PPO-catalyzed quinines (Qiao and al., 2021).

To prevent and/or attenuate the browning phenomenon, various procedures have been developed, namely the use of antioxidant solutions aiming at either inactivate PPO and POD or to avoid contact between the enzyme and its substrate. Antioxidant solutions have been traditionally employed since they were found to be the most effective in controlling browning, such as ascorbic acid and its derivatives and sulfites. Though, regardless of their efficacy, there is an increasing consumer demand for synthetic compounds' replacement by natural compounds (Moon and al., 2020).

Phenolic compounds display strong antioxidant activity and therefore potential as oxidative enzyme inhibitors. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Dias and al., 2020).

Inactivation of PPO and its relationship with phenolic compounds, it has been studied in apricots, apples, grapes, tea leaves, potatoes, lettuce, coffee, black raisins, anthocyanidins from strawberries, catechins, quercetin, shrimps, and others (Baltas and al., 2017).

Conversly, our results are allowed us to demonstrate the no inhibitor effect of by-products extracts for Deglet Nour variety for browning enzymatic. Yet, the extracts exhibited high phytochemical variability and source potentiel of phenolic compounds to be applied as browning mitigators. This can be explained by the chemical nature and structure of these compounds.

CONCLUSIONS

Enzyme activity (PPO and POD) and phenolic content are vital contributors to enzymatic browning different fruits during postharvest period and the storage process. Since enzymatic browning causes deterioration of sensory and nutritional quality and affects appearance and organoleptic properties, inactivation of PPO and POD is desirable for preservation of foods.

The effect on the PPO and POD enzymes (from dates) of the three by-products of date palm has been tested. The contents of phenolic compounds of hydro-methanolic extracts of by-products of date palm from Deglet Nour variety from the Ghardaïa region in the South of Algeria were evaluated for the first time. It was demonstrated that the hydro-methanol extracts of by-products of date palm contain a considerable quantity of phenolic compounds.

Moreover, the inhibition studies suggest that all extracts are not be good inhibitors for PPO and POD activities of Deglet Nour fruits. Therefore, no significant differences were observed for PPO and POD activities when the extracts of by-products of date palm were used.

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