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Full Length Research Paper

Effect of heat treatment on polyphenol oxidase and peroxidase activities in Algerian stored dates

KHALI Mustapha[1,2]*, SELSELET-ATTOU Ghalem[1]

[1]Laboratory of Food Technology and Nutrition, Mostaganem University, Algeria.
[2]National Institute of Agronomy, Algiers, Algeria.

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Effect of heat treatment (55°C/20 min) on polyphenol oxidase (PPO) and peroxidase (POD) activities and total phenolic compounds was investigated in Algerian dates (Deglet Nour variety) at Tamar (fully ripe) stage and in dates stored for 5 months at ambient temperature and in cold storage (10°C). Results obtained showed a high percentage of total phenolic compounds. These compounds decreased weakly as the fruit advanced in storage at ambiant temperature, whereas, they are still higher in heat treated and cold stored dates. Heat treatment did not show a significant effect on phenolic compounds variation. Deglet Nour dates showed that there was a decrease in both POD and PPO activities during storage for either heat treated and non-treated dates samples. Whereas, PPO activity was weaker in chilled dates comparatively to those stored at ambient temperature. Peroxidase activity showed a similar trend where a decrease was observed in all samples during storage, particularly in heat treated dates. In all cases, heat treatment seems to benefit reducing POD activity, whereas a relative stability in PPO activity was noted.

Key word: Date Deglet Nour, heat treatment, storage, phenolic compounds, peroxidase and polyphenol oxidase activities.

INTRODUCTION

Algeria is one of the most important date producing countries with an annual production of about 400.10^3 tonnes of dates of which Deglet Nour variety represents 50%, and are very appreciated by consumers (Anonymous, 2002). The deterioration of dates in storage is a major problem and results in undesirable changes in appearance, taste and food value of the fruit (Mark, 1941). Browning reactions are widely distributed, and constitutes along with other organoleptic properties, the basis of food acceptability. These reactions are one of the most serious alterations of date in storage and play a major role in marketing of dates locally and internationally. They could be attributed to enzymatic and non enzymatic reactions.

Polyphenol oxidase (EC 1.14.18.1) catalyses enzymatic browning through its action on mono and o-diphenols (Mayer and Harel, 1979; Golbeck and Camarata, 1981; Mayer and Harel, 1991). Browning of dates was found to be related to enzymatic and non - enzymatic reactions which take place during development, handling and processing (Al-bekr, 1972). PPO activity has been reported in many fruits, however, date has not received similar attention and few studies were reported (Hasegawa and Mayer, 1980; Hamdan, 1975; Benjamin et al., 1979; Mutalk and Mann, 1984). Previous studies have shown the major role of phenolic conpounds in defense mechanisms of plant tissues in response to infections or injuries (Mayer and Harel, 1991; Gerhard, 1993). However, little is published on the phenolic profile of the ripe date fruit (Lorente and Ferreres, 1988; Regnault-Roger et al., 1987; Mansouri et al., 2005).

A definitive role of peroxidase (EC 1.11.1.7) in plants has eluded plant scientists so far. There have been numerous reports in the literature with respect to their general involvement in oxidation of molecules at the expense of hydrogen peroxidase. This enzyme is probably appearing under the largest number of isoforms in plants. However, it is difficult to ascribe a particular role in single isoenzyme in the physiological events. In addition, plants peroxidases showed specific action with respect to different substrates (Siegel and Siegel, 1970)

*Corresponding author. E-mail: khali_mus@yahoo.fr.

and the activities changes in response to a wide range of environmental stimuli (Siegel et al., 1982; Gaspar et al., 1983; Petit, 1986; Castillo and Greppin, 1986). Peroxidase is recognized to be one of the most heat stable enzymes in plant and its resistance to heat has been reported by a numerous workers (Müftügil, 1985; Mc Lellan and Robinson, 1987). Peroxidase has been reported to be high during early fruit growth period when most metabolic activities are at the maximum. The review of literature shows that there are only few reported references about the POD of dates. Blanching is frequently used to destroy the enzymes in foodstuffs; care has to be taken when using water or steam blanching in order to prevent leaching of nutrients and flavour compounds. The phenolic compounds are responsible for the astringent taste of the fruit and act as substrate for the polyphenoloxidase and this enzyme is responsible of undesirable colours, flavour and texture during processing and storage of dates (Al Ogaïdi and Mutlak, 1986).

Recently, many studies on quality extension of fresh intact fruits using heat treatment have been carried out by several researchers. Exposure of fresh commodities to heat shock temperature (higher than normal ambient temperature) results in the modification of physiology and in many cases, interruption of synthesis of normal cellular protein the enzymes, and inactivation of the enzymes related to quality loss in fruits (Brodl, 1989; Klein and Lurie, 1990). Mutlak and Mann (1984) and Reynes (1997) reported that microwave treatment of dates was efficient in the inactivation of polyphénols oxidase and peroxidase, with only short periods of treatment compared with long period of blanching in boiling water to inactive enzymatic activities. This investigation was, therefore, carried out to study the effect of heat treatment on activities levels of PPO and POD and total phenolic compounds that occurs in Deglet Nour date variety stored in cold at 10°C for 5 months.

MATERIALS AND METHODS

Dates (Phoenix dactylifera L.) variety of Deglet Nour (Tamar stage-fully ripe) used in this study were obtained from the palm trees of the region of Tolga in Biskra Department (south-east. of Algeria). The samples were sorted and packed in polyethylene bags and stored at +4°C in a freezer until the beginning experimentation.

Heat treatment of dates samples was carried out in a chamber at 55°C ±1°C for 20 min, and then stored for 5 months in cold at 10 ± 1°C (75-80% R.H). The heat treatment room comprises a chamber (1000 mm long x 100 mm wide x 400 mm high) with a maximum measurable fruit capacity of 10 kg as set up, through which conditioned air is circulated down wards by a high capacity fan. Conditioning of air to the required temperature and humidity occurs in a plenum adjoining the treatment space. The treatment is calibrated at the maximum intended temperature against a certified standard mercury thermometer in a precisely standard water bath. Fruits were hold in stain less steel drawers during treatment. When treatment is completed, fruit is cooled by aeration with ambient air. Comparative shares were stored in ambient temperature. At the end of each month of storage, date samples were stored at -18°C in a deep freezer to stop enzymatic reactions. Before analyses, the perianths and seeds were removed and date meat was cut into small pieces and mixed well. All the preparative procedures were carried out below 5°C.

Total phenolics determination

Total phenolics were extracted as described by Brenes et al. (1992) and determined on 5 g of fresh dates according to AOAC (1985) method. Samples were mixed with 80 ml of aqueous methanol (800 ml/l) and 20 ml of 5 g/l metabisulfite, homogenized for 30 s and left for 15 min at 4°C. The homogenate was filtered on Büchner prior to analysis. Total phenolics of extracts were quantified colorimetrically at 730 nm with a PUY Unicam spectrophotometer (SP 9000 model), using chlorogenic acid (Sigma Chemical Co.) as a standard.

Preparation of PPO extract

Acetone powders were prepared according to the procedures described by Wong et al., (1971). Frozen dates were pitted and homogenised in a blender with 150 ml of cold acetone (-15°C) and 3.0 g of polyvinyl pyrrolidone for 2 min. The slurry was filtered on a sintered glass funnel and washed with cold acetone under low vacuum until dry uniform powder was obtained. The powder was extracted with 0.25 M potassium phosphate 0.03 M ascorbic acid pH 7.0 in ration 1:5 (w/v) for 2 min in a blender. The slurry was centrifugated at 21 000 x g for 30 min at 4°C and then the supernatant was assayed for PPO activity.

PPO activity assay

PPO activity was determined according to the procedure described by Rivas and Whitaker (1973). The reaction mixture contained 0.6 ml of 0.01 M catechol in 0.01 M potassium phosphate buffer, pH 6.2. The reaction was carried out in a spectrophotometer tube at 30°C and it was initiated by according 0.05 – 0.20 ml of enzyme solution (volumes were adjusted to 6.2 ml with 0.01 M potassium buffer). Catechol was used as a substrate for determining polyphenoloxidase activity. PPO activity was followed by according the absorbance at 30 s intervals for a period of 3-5 min with a spectophotometer PUY Unicam (SP 9000 model) at 420 nm. One unit of PPO activity is defined as the amount of enzyme which causes 0.01 increases in absorbance per minute under assay conditions.

Preparation of POD extract

Samples (10 g) were mixed with 100 ml of 0.05 M potassium phosphate buffer pH 7.0. The mixture was centrifugated at 20 000 x g for 15 min and the supernatant was assayed for POD activity (Wahid, 1980).

POD activity assay

Peroxidase activity was determined by measuring the colour development at 400 nm in enzyme extract mixed with hydrogen peroxide and gaiacol. 1 ml of enzyme extract was mixed with 1 ml of 0.5% of gaiacol, 1 ml of 0.5% of hydrogen peroxide and 18 ml of sodium phosphate buffer at pH 6.5. Colour development was measured by UV spectrophotometer (PUY Unicam, SP 9000 model) as a change in absorbance of 0.001/min. All the experiments were replicated twice and the analyses were carried out in duplicate for each replication.

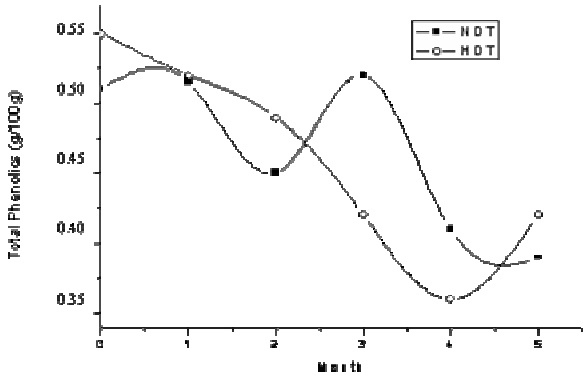

Figure 1. Total phenolics changes in heat treated and nontreated dates during storage at ambient temperature.

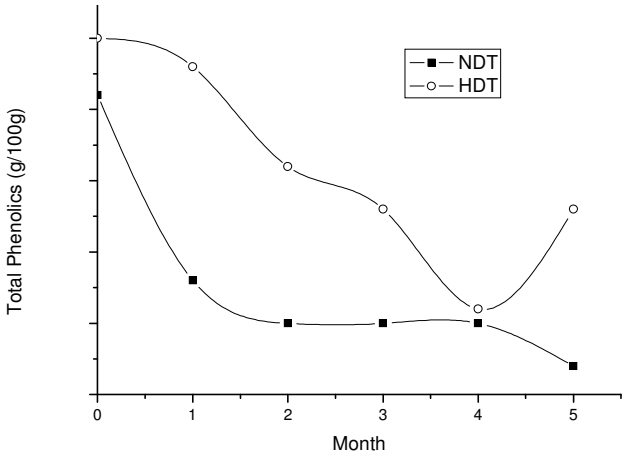

Figure 2. Total phenolics changes in heat treated and non-treated dates during storage at 10°C.

RESULTS AND DISCUSSION

Phenolic compounds

Results of phenolic compounds are reported in Figures 1 and 2. The date Deglet Nour variety contains a high percentage of these compounds (510 mg/100 g FW) which decrease under heat treatment at 55°C/20 min as shown in Figure 1. Heat treatment seems not to affect the total phenolic fraction and these compounds do not decrease significantly under heat treatment at 55°C/20 min. During storage at ambient temperature, the phenolic compounds in non-treated dates (NTD), decreased gradually for the 5 months of storage. The same trend was noted in heat treated dates (HTD) stored in the same conditions, though the phenolic compounds content was slightly lower. These results show that total phenolic compounds decreased in both treated and non treated dates at the storage at ambient temperature and reached a minimum content at the fourth month (360 and 390 mg/100 g FW). This decrease may be due to the conversion of soluble tannin into insoluble tannin (Mutlak and Mann, 1984) and also to enzymatic oxidation of disappearance of flavan and caffeoyl shikimic acid during the maturity stage (Maier and Metzler, 1965b; Maier, 1964) as it can be due to the activity of polyphenol oxidase during the heat treatment. Total phenolic compounds in dates are responsible for the astringent taste of the fruit and this taste is related to the presence of soluble tannin. The phenolic compounds take part in the enzymatic and non enzymatic oxidative browning of date and result in undesirable changes in appearance, tatste and food value of the fruit (Mutlak, 1980).

The influence of storage at 10°C on total phenolic compounds is showed in Figure 2. The same trend in reduction of the phenolics content was noted in both treated and non-treated dates, although this content was lower in the non treated dates (NTD) comparatively to the heat treated samples (HTD) during the whole storage period time. The lowest contents were reported at the fifth month of storage (320 and 440 mg/100 g FW).

An important differences in phenolics content are observed during storage at 10°C between the heat treated and the nontreated dates in opposite of either treated or non-treated dates stored at ambient temperature. For all samples stored in cold, stability was noted very early; practically from the third month for the NTD and the HTD dates samples with an average content of 330 mg and 440 mg/100 g FW, respectively for the NTD and HTD. In conclusion, there is always a reduction in the phenolics compounds during storage. Heat treatment seems acting by a stabilisation of the phenolics content of dates, this trend is more observed in dates samples stored in cold.

Enzymatic activities

The effects of heat treatment (55°C/20 min) on enzymatic activities have been shown in Figures 3, 4, 5 and 6. Polyphenol oxidase (PPO) activity was present in all months for samples examined (Figure 3 and 4). The effects of heat treatment on PPO activity have showed the applied temperature caused an increased velocity of reaction between the enzyme and the substrate (Maier and Shiller, 1960). This can be seen in the changes in soluble phenolic compounds which constitute the substrate of these enzymes.

The results show that during heat treatment for 20 min at 55°C, the enzyme was partially inactivated. PPO has been reported inactivated by blanching in hot water at 100°C for 1.5 min (Rashid, 1950). During storage, decreasing trend in PPO activity is observed either at ambient temperature or at 10°C. The lowest values of activities are noted at the fifth month of storage where activities of 0.100 UI and 0.050 UI are registered at ambient temperature for the NTD and HTD samples, respectively. In cold, the levels of PPO activities still very near from those noted at ambient temperature. The applied cold (10°C) is

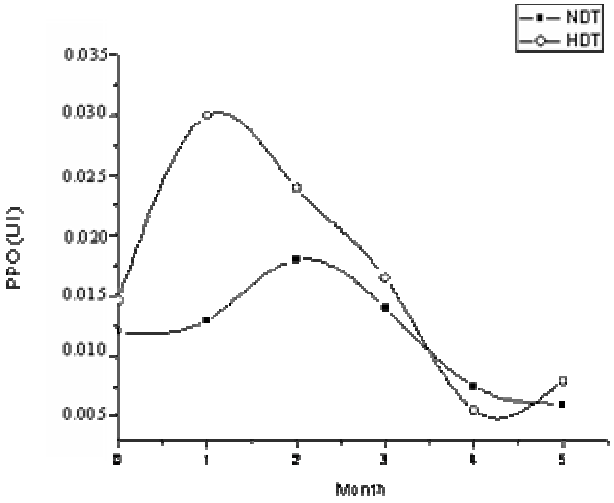

Figure 3. Polyphenoloxidase changes in heat treated and nontreated dates during storage at ambient temperature.

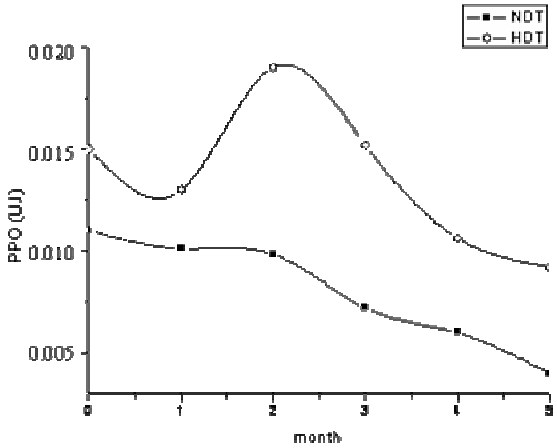

Figure 4. Polyphenol oxidase changes in heat treated and non treated dates during storage at 10°C.

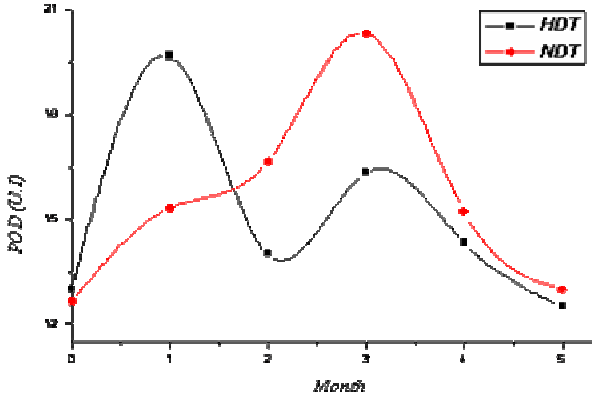

Figure 5. Peroxidase changes in heat treated and non treated dates during storage at ambient temperature

not enough to induce enzyme inactivation.

For POD enzyme, Figures 5 and 6 show that heat treatment at 55°C/20 min has no apparent effect POD activity and the activities still the same before storage. POD activity was fluctuating without reaching alarmous levels during the storage either at ambient temperature or in cold (10°C) and the lowest activities are noted at the last month of storage (5th month). For Maier and Shiller (1961), peroxidase activity is less active than polyphenol oxidase during storage or after ripening of dates and therefore, has no effect on the darkening of dates as reported by Rashid (1950). The applied heat treatment was not enough to inactivate the peroxidase activity which is relatively stabilized during storage particularly at 10°C. Inactivation of PPO is important to prevent excess

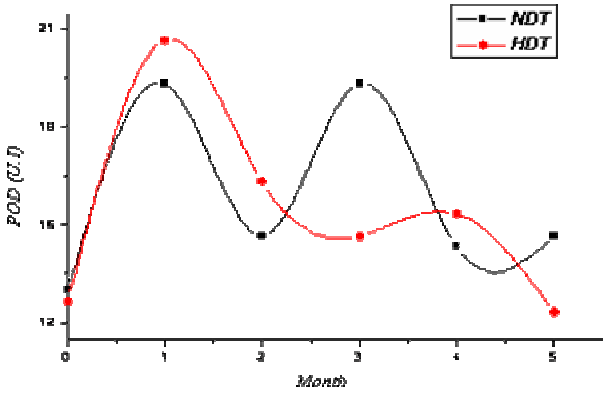

Figure 6. Peroxidase changes in heat treated and non treated dates during storage at 10°C.

darkening but complete inactivation of POD may not be essential. Other workers reported that peroxidase was inactivated at 100°C for 14 min (Rashid, 1950).

Conclusion

Numerous treatments have been used to control post harvest damage and undesirable enzymatic browning reactions in food products. To prevent or reduce this phenomenon, heat treatment of dates constitute a promising processing technique which was found efficient in the inactivation of polyphenol oxidase and peroxidase and in reducing or stabilizing total phenolics which are the substrate of these enzymes. The period time of 20 min was necessary to inactivate the enzymes and still non aggressive compared to others treatments such blanching in boiling water. Used at the correct dosage, this treatment appears to be very useful method in controlling deteriorative changes in dates during subsequent storage. Such measure is not a substitute for the cold storage to maintain the quality as shown by the best behaviour of the dates stored at 10°C.

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