ISSN : 0974 - 7508 Volume 6 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 6(4), 2010 [205-212]

Preliminary analysis of antioxidant activity and colour of water soluble extract from Tunisian Deglet Nour and Allig palm date seeds

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Received: 25th November, 2010 ; Accepted: 5th December, 2010

ABSTRACT

The antioxidant activity and phenolic content of water soluble extracts (WSE) of date seeds from Deglet Nour and Allig varieties were studied. Total phenolic contents in WSE of Deglet Nour and Allig seeds were, in dry weight, 38.34 mg GAE /g and 37.78 mg GAE /g, respectively. Free radical scavenging activities of WSE were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH). WSE pigments showed an excellent DPPH radical scavenging activity with 75-78% of inhibition. The variation in colour of WSE pigments of date seeds have been evaluated over pH range 2-10. The study was made on crude extract. The pigment directly from Deglet Nour and Allig date seeds can be dissolved in alkaline and acidic waters solutions. The WSE pigments of date seeds showed much redder colour at alkaline solution than the acidic solution. The colour was reversible with pH variation. No significant difference between the UV-Visible absorbance of the Deglet Nour and Allig seed WSE. When varying pH from acidic to alkaline values, a progressively apparition of two peak in the visible wavelength was observed while no noticeable change was observed in the UV absorbance wavelengths for the two studied varieties. A concomitant increase in the intensity of the UV absorption wavelength was also observed for Deglet Nour and Allig seeds. The red-brown taint obtained in the neutral and alkaline pH values may allow the studied WSE pigments of date seeds compound to be regarded as a possible colorant for some slightly red-brown coloured neutral and alkaline products.

KEYWORDS

Phoenix dactylifera L.;
Date seeds;
Penolic compounds;
Antioxidant;
Natural pigment;
Colour.

INTRODUCTION

Nowadays, the consumption of fruit and vegetables is regarded as important and good for health. Fruit seeds intake is associated with a reduced risk of many diseases, including cancers[1,2] and cardiovascular disease[4,5]. The therapeutic effects of the polyphenolic compounds have been attributed to their antioxidant properties. Recently, polyphenolic constituents of various seeds have been reported to contain potential medicinal properties including antioxidant activities[6-9]. Phenolic compounds of fruit seeds mainly phenolic acids

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and flavonoids. Therefore, the study of phenolic acids, flavonoids and high molecular tannins as natural antioxidants has greatly increased.

In addition, colour is an important factor in the acceptability of food product[10]. Natural colorants have attracted widespread interest because of their general health and safety image and numerous other important properties. Besides, their eventual potential health effects are attracting considerable interest in the international scientific community. The development of natural food colorants from natural sources is thus receiving increasing attention as natural pigments tend to replace synthetic ones.

The extraction of antioxidants and coloured pigments from natural sources is desired, since these bioactive substances are often used in functional foods, food additives, nutraceuticals, pharmaceuticals and cosmetic industries[11].

In North Africa and Arabian countries, date palm (*Phoenix dactylifera* L.) is the major fruit tree and an economically important crop for many populations. In Tunisia, the annual date palm production was estimated to be 127 000 T per year with dominance of the Deglet Nour variety constituting about 60% of the total production[12]. This variety has a very good sensory quality and a high commercial value. In Tunisia, Deglet Nour and Allig are the most consumed varieties[13].

Date palm fruit is composed of a fleshy pericarp and seed[14-16]. The fleshy part of dates always plays an essential role in the diet, leaving a large quantity of seeds as the waste product from dates. Date seeds are a by-product of date processing; it is known that the average weight of date seeds ranges from 10% to 15% of the date's weight[17]. Date seeds are used in the feeding of ruminant animals. The chemical composition was: Crude protein, crude fat, crude fibre and ash range 5-7%, 4-10%, 12-27% and 1-2%, respectively[16,18-22].

This by-product of date processing industries could be regarded as an excellent source of food ingredients with interesting technological functionality that could also be used in food as an important source of dietary fibre and polyphenolic compounds[16,21-27].

In the previous research, the chemical composition of the water soluble extract from Deglet Nour and Allig seeds had been studied in our laboratory by Bouaziz et al.[27]. The good nutritional values of the water soluble extracts are based on their dietary fibre (80-83%) and protein (14-17%) contents[27]. However, the phenolic compounds, antioxidant activity and colour of this water soluble extract from the tow studied date seed varieties Deglet Nour and Allig have not been reported. The objective of the present study was to explore the feasibility of using date seeds from the most produced and consumed Tunisian varieties: (Deglet Nour and Allig) through the extraction of coloured pigments, polyphenol compounds and to evaluate their antioxidant activities.

EXPERIMENTAL

Material

The seeds of the two cultivars under investigation (Deglet Nour and Allig) were directly isolated from 50 kg of date fruit from Degach region (Tunisia), collected at the ''Tamr stage'' (full ripeness) and kept at 10°C for a week. The seeds were soaked in water, washed to get rid of any adhering date flesh, and then air-dried. They were also further dried at about 50 °C. Date pits, of each variety, were separately milled in a heavy-duty

TABLE 1 : Antioxidant activities and phenolic compounds of the two studied date seeds

Seed Varieties	Total phenolic compounds (mg of GAE/g)	Antioxidant activity (% inhibition of DPPH)
Deglet Nour	38.34^{a}±1.07	78.49^{a}±1.67
Allig	37.78^{a}±0.82	75.28^{a}±0.88

Values in rows with different letters are significantly different ($p \leq 0.05$)

TABLE 2 : CIE Lab parameters (L*, a*, b*) of WSE from the two studied date seeds

Para-meters	WSE Deglet Nour seeds			WSE Allig seeds		
	*L**	*a**	*b**	*L**	*a**	*b**
pH2	57.11^{a}±0.90	4.84^{a}±0.02	24.40^{a}±0.32	57.06^{a}±1.20	4.65^{a}±0.32	22.61^{a}±1.45
pH3	56.38^{a}±1.46	2.37^{a}±0.03	22.35^{a}±0.35	56.31^{a}±2.42	2.62^{a}±0.08	20.41^{a}±0.74
pH4	54.15^{a}±1.96	5.66^{a}±0.35	$25.^{87a}$±1.08	53.34^{a}±1.83	6.73^{a}±0.15	23.77^{a}±0.60
pH5	46.44^{a}±3.82	10.48^{a}±0.97	29.18^{a}±3.70	50.32^{a}±1.02	10.13^{a}±0.12	27.60^{a}±0.40
pH6	42.33^{a}±1.37	19.44^{a}±0.44	30.57^{a}±0.88	45.22^{a}±0.85	17.78^{b}±0.14	31.11^{a}±0.22
pH7	44.51^{a}±0.72	19.24^{a}±0.16	29.80^{a}±0.30	44.67^{a}±0.12	20.70^{b}±0.38	29.77^{a}±0.83
pH8	40.17^{a}±1.19	25.29^{a}±0.44	35.02^{a}±0.74	41.75^{a}±2.04	22.70^{b}±0.81	28.06^{b}±1.15
pH9	33.14^{a}±1.31	29.02^{a}±1.04	37.65^{a}±2.27	43.45^{b}±1.99	20.96^{b}±0.55	23.23^{b}±0.58
pH10	30.30^{a}±1.57	26.83^{a}±1.47	32.89^{a}±2.72	37.55^{b}±0.86	25.97^{a}±0.44	30.68^{a}±0.60

Values of same parameter in lines with different letters are significantly different ($p \leq 0.05$)

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grinder and then preserved at - 20 °C until analyses.

Lipid extraction was carried out as described by Besbes et al.[16] with a SER 148 Solvent Extractor (Velp Scientifica, Italy) equipped with 6 Soxhlet posts. The extraction was carried out over a 30 min, with thimbles immersed in boiling petroleum ether, and 60 min of reflux washing.

Methods

(1) Preparation of water soluble extract from defatted date seeds

The water soluble extract from defatted date seeds was prepared according to the Tsaliki, Kechagia and Doxastakis methode[28].

The produced flour of defatted date seed was mixed with distilled water (1:10 w/v), adjusted to pH 10 with NaOH and after stirring for at least 40 min was centrifuged (Backman) at 4000 rpm for 20 min. The residue was again mixed with distilled water (1:5 w/v), readjusted pH 10 and centrifuged follwing the same process. The supernatants of both centrifugations were blended: Water soluble extract (WSE) and used for the colour studies (Figure 1).

(2) Preparation of extract from defatted dates seeds and determination of total phenolic content

Defatted dates seed samples (200 mg) were extracted with MeOH: H2O (4:1 v/v; 5 ml) for 24h at room temperature according Djeridane et al.[29] with same modifications. After removal of methanol under reduced pressure in a rotary evaporator at 40°C, the aqueous solution was extracted with ethyl acetate. Then the ethyl acetate fraction was evaporated to dryness using a rotary evaporator. The precipate was dried, dissolved in 5ml of absolute methanol and kept at -20°C.

The pH was measured at 20°C using a MP 220 pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

(3) Analysis of the total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method described previously with a little modification[30]. Briefly, the appropriate polyphenolic extracts from Deglet Nour and Allig seeds were oxidized with 0.2 N Folin-Ciocalteu

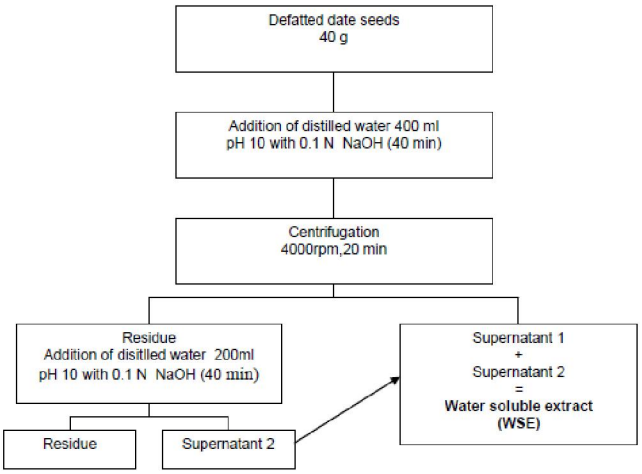


Figure 1 : Laboratory production of date seed water soluble extract (WSE)

reagents and then the reaction was neutralized with saturated sodium carbonate (20%). The absorbance of the resulting blue colour was measured at 765 nm with a UV 2401 PC SHIMADZU (Kyoto, Japan) spectrophotometer after incubation for 2 hr at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as mg of gallic acid equivalent (GAE) per g of dry weight (DW).

(4) Radical scavenging activity (RSA)

The free radical scavenging activity of date palm syrup was estimated according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method proposed by Turkmen et al.[31] with some modifications. A 100 µL aliquot of sample was mixed with 2 mL of 0.1 mM DPPH radical in methanol and let to stand at room temperature in the dark. Absorbance at 517 nm was measured, sixty minutes later, using a UV 2401 PC -Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). Methanol was used as a blank and distilled water as a control instead of samples.

Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$AA\% = \left[\frac{(A_1 - A_2)}{A_1}\right] \times 100$$

where A_1 is the absorbance of the control reaction and A_2 is the absorbance in the presence of the sample. The experiment was carried out in triplicate and the results are mean values.

Full Paper

(5) Measurements of colour

The CIE Lab parameters (L*, a*, b*) were directly read with a spectrophotocolorimeter MS/Y-2500 (Hunterlab, In., Reston, VA, USA), calibrated with a white tile. In this coordinate system, the L* value is a measure of lightness, ranging from 0 (black) to 100 (white); the a* value ranges from - 100 (green) to + 100 (red) and the b* value ranges from - 100 (blue) to + 100 (yellow).

(6) Spectra absorption

The WSE of date seeds was diluted (1:2) and adjusted at different pH range from 2 to 10. These solutions were transferred to sample tubes. UV–Vis spectra were recorded between 250 and 700 nm on a 2401 PC -Shimadzu spectrophotometer (Kyoto, Japan) equipped with Scan Master manager software and a computer unit. Pure water was used as reference cell solutions. Each experiment was conducted in triplicat.

(7) Statistical analysis

Duncan's Test, at the level of $P \leq 0.05$, was applied to data to establish significance of difference between the samples. Statistical analyses were performed on statistical analysis package STATISTICA (Release 5.0 Stat Soft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Total phenolic content

The total phenolic content of Deglet Nour and Allig seeds was relatively high for all seed extracts tested (TABLE 1). WSE of Deglet Nour seeds contained comparable total phenolic content (38. 34 mg GAE/g) than those from WSE of Allig seeds (37.78 mg GAE/g). This phenolic content was recorded for these from oman date seeds studied by Al Farsi et al.[32] (31.02-44.30 mg GAE/g). Although phenolic compounds are found in virtually all plants and plant parts, their quantitative distribution varies between plant species, and between different organs in a plant[33]. Plant seeds with high levels of phenolic compounds have been shown to exhibit high antioxidant capacities.

Similar results were reported by Siddhuraju[34] that methanolic extracts of raw and dry heated seed coat of *Tamarindus indica* shoots had high levels of phenolic compounds (32.96 mg GAE/g) and corresponding potent antioxidant activities). Also, Soong and Barlow[35] showed that the total phenolic content in extracts of avocado, longan, mango and tamarind seeds were 88.2, 62,6, 117 and 94.50mg GAE/g respectively. These values of phenolic content were higher than these of date seeds. Alu'datt et al.[36] (2010) reported that the The total phenolic content of olive cake extract ranged from 2.07 to 3.80 mg of GAE/g, while these of date seed WSE ranged from 37.78 to 38.34 mg of GAE/g.

Scavenging activity on DPPH radical

The DPPH radical assay could determine the radical scavenging activities of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm and this assay possesses the advantage of rapid, facile and commercially available[37]. A concentration-dependent assay was carried out with WSE and the results are presented in TABLE 1. These results provide a direct comparison of the antioxidant activity of the two studied date seed varieties. WSE possessed significant scavenging activity on the DPPH radical and acted as an excellent antioxidant.

The scavenging activity was ranged from 75% to 78% .WSE of Deglet Nour seeds showed a slightly higher scavenging activity than those of Allig seeds (78.49±1.67 % against 75.28^{a}±0.88%).

Colour variation of WSE of date seeds at different pH values

TABLE 2 presents the CIE Lab (L *, a * and b *) parameters of WSE from date seeds. No significant differences was observed for a* value of the tow WSE ($P \geq 0.05$); both having a brown colour (Figure 2 and 3). WSE from Allig variety was characterised by a slightly higher L * and lower a* values at pH 8 to 10 compared to the Deglet Nour WSE. This suggests that this latter is slightly darker in alkaline pH. This could be explained by a better extraction of the pigments responsible for the dark colour at alkaline pH. Bouaziz et al.[38] reported that the Allig seeds were darker than Deglet Nour seeds.

Indeed, for example, at pH 9 a * and L* of Deglet Nour seed WSE is 29.02±1.04 and 33.14±1.31 against 20.96±0.55 and 43.45±1.99 for Allig seed WSE, respectively. The WSE of date seeds is true natural dyes

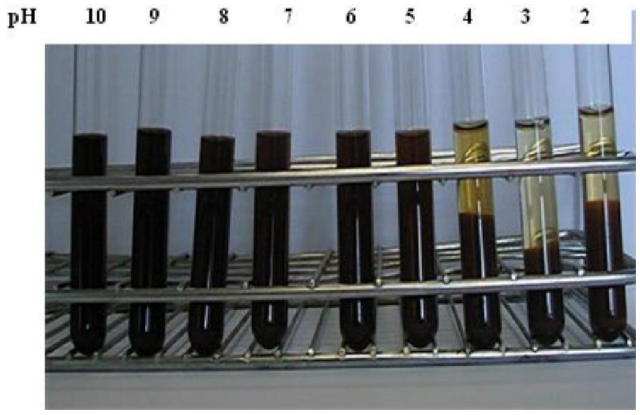


Figure 2 : Effect of pH variation on WSE colour of defatted Deglet Nour seeds. WSE: water soluble extract

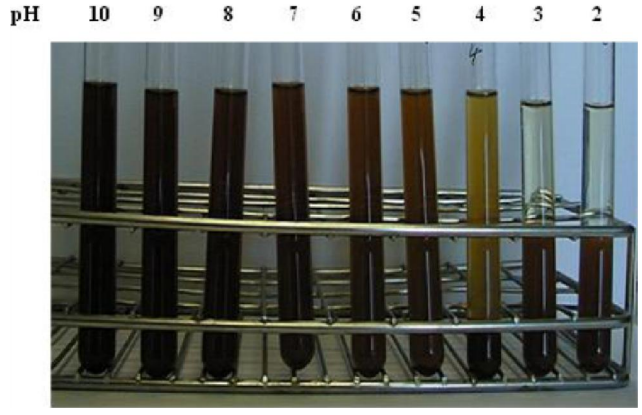
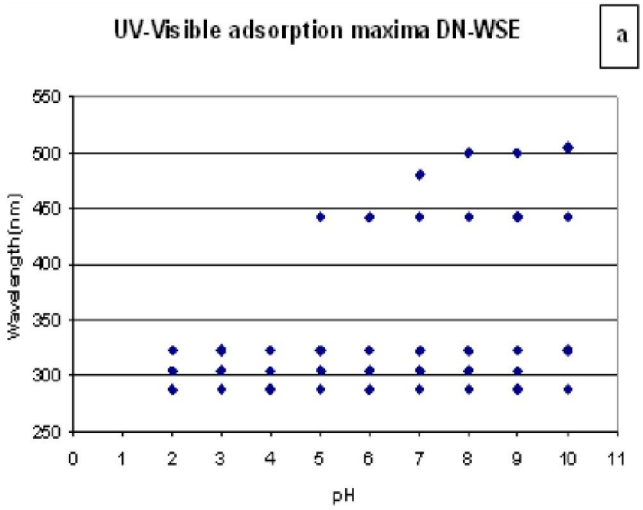


Figure 3 : Effect of pH variation on WSE colour of defatted Deglet Nour seeds. WSE: water soluble extract



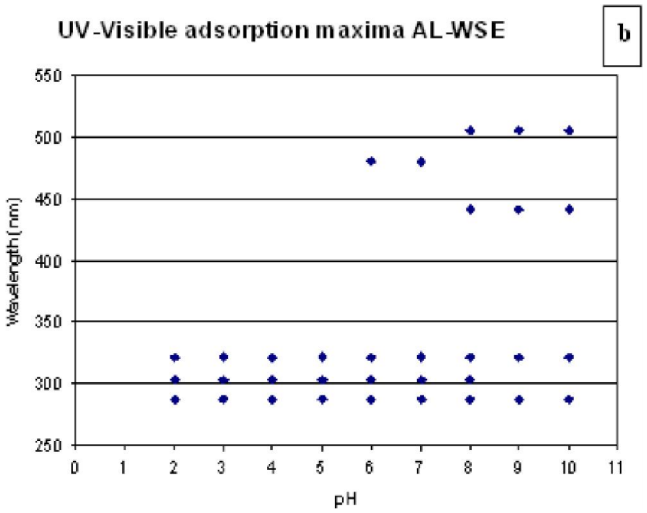


Figure 4 : UV–Visible absorption maxima at different pH values for the WSE pigments of defatted (a) Deglet Nour seeds and (b) Allig seeds. WSE: water soluble extract, DN: Deglet Nour, AL: Allig

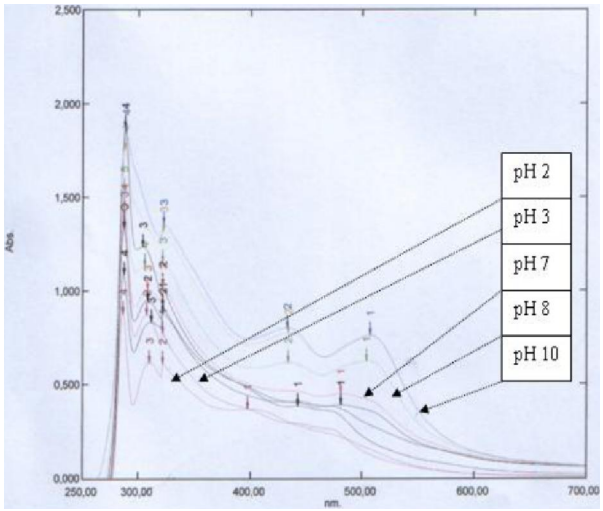


Figure 5 : UV–Visible spectra of the WSE Allig seeds recorded at different pH values: from 2 to 10. WSE: water soluble extract

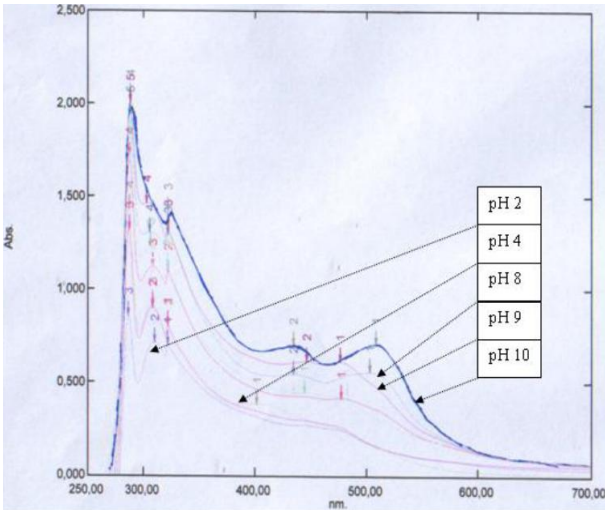


Figure 6 : UV–Visible spectra of the WSE Deglet Nour seeds recorded at different pH values: from 2 to 10. WSE: water soluble extract, Number presented the absorption maxima

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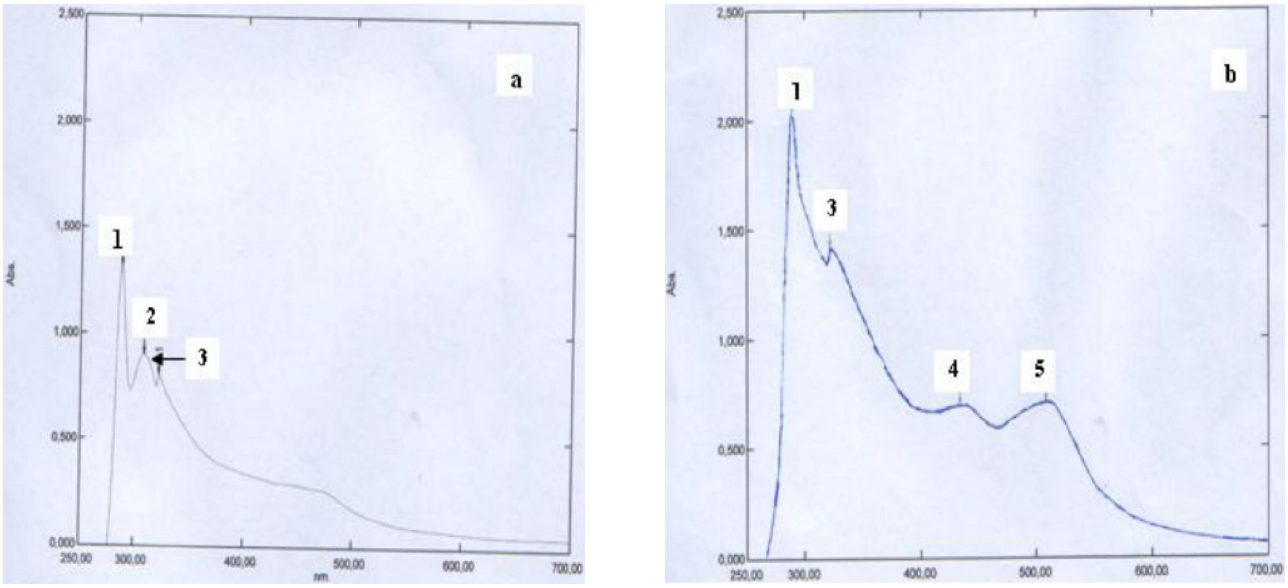

Figure 7 : UV–Visible spectra of the WSE Deglet Nour seeds recorded at (a) pH 2 and (b) pH 10. WSE: water soluble extract, Number presented the absorption maxima

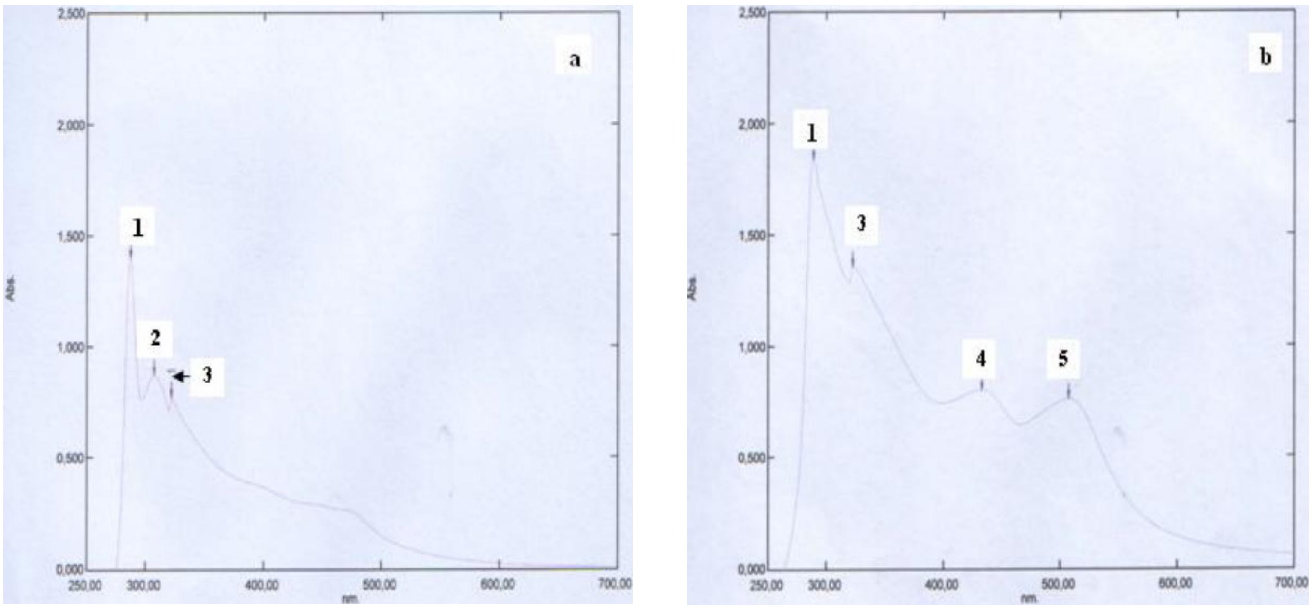

Figure 8 : UV–Visible spectra of the WSE Allig seeds recorded at (a) pH 2 and (b) pH 10. WSE: water soluble extract

and by consequence their incorporation in the foodstuffs has an effect on the colour of the finished product.

The spectrophotometer profils of the two studied WSE from Deglet Nour and Allig seeds were represented in figure 4, 5, 6, 7 and 8. At pH 10, UV-Visible spectrums of the WSE of date seeds were characterized by the presence of four absorption maxima (Figure 4a and 4b), the first major and the second minor absorption maxima in the UV region located around 288 nm and 322nm, respectively (Figures 5, 6, 7a, 7b, 8a and 8b).

In addition, two other less intense maximum located at 442 nm and 505 nm were also observed in visible region.

At pH 2 to 9, for Deglet Nour seed WSE, the UV-Visible spectrum was characterized by the presence of absorption maxima at 304 nm (Figure 4a), for Allig seed WSE, the same maxima absorption (at 304nm) was appeared at pH 2 to 8.

When decreasing pH from alkaline to acid values,

the visible absorption maxima for WSE of date seeds despaired for pH 6-5 to 2 (Figure 4a and 4b) and the shape of the spectrum was unchanged but a steady decrease of the absorption intensity was registered, as can be seen in figure 5, 6, 7a, 7b, 8a and 8b).

For Deglet Nour seed WSE, from pH 2 to 4, three maxima of the UV absorption were observed (Figure 4a). In addition, at pH 5 to 10, the maximum at 442 nm was also observed. At pH 7, a new maximum was detected at 480, a shoulder at 505 nm at pH 8, 9 and 10 and to stabilise a two visible absorption maxima. From pH 2 to 10 a gradual increase of the maximum absorption at 288 nm was observed, concomitant with the appearance of the maximum located around 442 nm and 505 nm as shown in figure 6.

For Allig seed WSE, an equivalent evolution was observed compared with these from Deglet Nour seed WSE (Figure 4b). Thus, for pH ranging from 2 to 5, three maxima of the UV absorption were recorded at 288, 304 and 322 nm. From pH 6 to 7, four maxima were observed, respectively, at 288, 304, 322 and 480 nm. At pH 8, five maxima were observed, respectively, at 288, 304, 322, 480 and 505 nm. Only the latter were observed from pH 9 to 10 except the maxima UV absorption at 304 nm.

In contrast, no noticeable variation was observed in the case of the absorption maximum around 288 and 322 nm which remained almost constant in the studied pH range (Figure 4a and 4b).

WSE was red-Brown colour from pH 5 to 10. This colour became light with pH decrease. At pH 2 a* was 4-5 against 25-27 at pH10. Darkness and redness of WSE could be due to the better extraction of red pigments of the date seeds. From pH 2 to 5, the WSE pigments precipitated. This precipitation increased with the decrease of pH. The pigment responsible of darkness and redness of WSE could be due to the possible liaison and interaction of different components (fibre, protein and pigment) and the precipitation of red pigments of date seeds at pH ranged 2-5. The decolourization of WSE pigments of date seeds can by explained by denaturation of protein and the colour development of the WSE was related to the protein conformation. These results was similar of these reported by Kouji et al.[39]. They explained that the splitting of the S-S linkage of DSP-modified phycocyanin molecule caused the reduction of colour development of phycocyanin under urea-treatment and the colour development of phycocyanin is closely associated with its high order structure of protein. Moreover, the reversibility of denaturation and renaturation of protein conformations can be due to in relation to their functions[39].

Due to the effect of pH, WSE can be used at the stable state in neutral and alkaline condition. Its property of changing colour with pH will help in the food processing and other applications. These results suggested that the WSE of date seeds can be considering a natural dye and their incorporation in food formulation has an effect on product colour.

CONCLUSION

The study aimed to characterise the WSE from date seeds for antioxidant activity. The results showed that WSE exhibited an excellent antioxidant activity. Furthermore, it is important to study date seed phenolic compound profiles that may contribute to antioxidant activity in this material.

Moreover, this work showed that the colour of the studied WSE from the two date seeds varieties was significantly influenced by pH. In relatively acidic aqueous medium, the pigment occurred as a lightness colour. From pH 5 the pigment colour intensity increased, while there was a gradual to more brown-reddish colour. The possible use of the studied pigment as a food colorant in slightly neutral and alkaline products should therefore be considered.

This work finally opens perspectives for further investigations of a number of other properties, such as temperature stability and the studied the relationships between colour and protein/ fibre, remain of a high interest and should be investigated.

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