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EFFECT OF VARIETIES AND PROCESSING ON CAROTENOIDS AND α-TOCOPHEROL CONTENT OF DATE PALM (*Phoenix dactylifera* L.) FRUITS

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ABSTRACT: The phytochemicals antioxidants; ß-carotene, lycopene and α-tocopherol contents of fruits of seven date (soft, semi-dry and dry) varieties were investigated. The results showed that Saidy date variety contained the highest ß-carotene amount (3970.0 µg/100g fresh weight) followed by Zaghloul fruits (2767.5 µg/100g fresh weight). While, Khudri fruits recorded the lowest ß-carotene value (80.0 µg/100g fresh weight). Data revealed that the different date varieties presented a lycopene content varied from 170.0 to 3695.0 µg/100g fresh weight for Manthour and Saidy date fruits, respectively. Also, it could be observed that the seven studied date varieties contained amounts of α-tocopherol ranging from 204.0 to 966.0 µg/100g fresh weights. Generally, there were highly significant differences in ß-carotene, lycopene and α-tocopherol contents (µg/ g dry weight) among all the studied date varieties; except between Samani and Amri (soft and semi-dry varieties, respectively) in their ß-carotene content. From the present results, every 100g of fresh date flesh will provide an adult human with 2.70 to 132.33% of his daily requirements of ß-carotene. However, the same weight of date will coverd only 1.02 to 4.83% of his α-tocopherol daily needs. The effect of technological processes on the studied phytochemical levels of Khudri date products were also studied. It was observed that the heat treatment during the preparation of Khudri date juices occurred an increase in ß-carotene (1.75 %) and α-tocopherol (126.65 %) levels compared with the raw fruits, while, lycopene content was decreased by 21.07 %. Besides, the processing of date syrup (Dibs) caused a more decreased in both ß-carotene and lycopene in the syrup compared

with the raw juice, while, α-tocopherol was not detected. The by-product; date fiber, had high level of α-tocopherol (5.26 µg/ g dry weight).

Key words: Carotenoids, α-tocopherol, antioxidants, date varieties, juice, syrup (dibs), processing.

INTRODUCTION

The fruit of the date palm (*Phoenix dactylifera* L.) has long been an important fruit for the native population from Western Persia across of the Arabian Peninsula and North Africa. It is considered a vital component of their daily diet. This fruit has great importance from nutritional and economical points of view (Mansouri et al., 2005). Worldwide dates production has increased exponentially over the last three decades. World production of dates was approximately 5.53 million tones in 2005 (FAO, 2006). Top five dates producer countries in 2005 (1000 tones) were Egypt (1,170), Saudi Arabia (970.49), Iran (880), United Arab Emirates (760) and Algeria (516.29). Dates represent an important source of export earning for some countries in Africa and the Middle East. Europe, in particular the European Community (EC), is a key market for date exporters. Although the EC imports of dates represent only 10% of world imports in volume, they account for some 30% in value (FAO, 2002).

Collectively vitamin E and ß-carotene are referred to as the antioxidant vitamins. All work both singly and synergistically to prevent or delay oxidative reactions that lead to degenerative diseases including cancer, cardiovascular diseases, cataracts and other diseases (Elliot, 1999). Recent epidemiological studies have consistently shown that there is a clear significant positive associations between intake of fruits and vegetables and reduced rate of heart diseases mortality, common cancers and other degenerative diseases as well as ageing (Garcia-Closas et al. 1999; Dillard and German, 2000; Prior and Cao, 2000; Van Duyn and Pivonka, 2000 and Wargovich, 2000; Block et al., 2001; Bazzano et al., 2002; Stacewicz-Sapuntzakis and Diwadkar-Navssariwala, 2004). The

strongest evidence is related to reduce risk of cancers of mouth and pharynx, esophagus, stomach and colon. More recently a core human study in European Union has demonstrated that increased consumption of carotene rich fruits and vegetables increased low density lipoprotein (LDL) oxidation resistance and higher plasma concentration of total carotene was associated with lower DNA damage and higher repair activity (Southon, 2000). This is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibers and other biotic compounds (Kaur and Kapoor, 2001). Lycopene has recently emerged as efficient singlet oxygen quencher (Levy et al., 1995). Lycopene, s ability to act as an antioxidant and as a scavenger of free radicals that are often associated with carcinogenesis is potentially a key to its beneficial effects on human health. It may prevent carcinogenesis and atherogenesis by interfering positively with oxidative damage to DNA and lipoproteins (Clinton, 1998). It may also inhibit the formation of LDL cholesterl's oxidized products, which in turn have been suggested to participate in the early stages of coronary heart diseases (Diaz et al., 1997). Therefore, recommendations have been made to increase daily intake of fruits and vegetables rich in these nutrients to low risk of cancer and cardiovascular diseases (Krichevsky, 1999; Prior, 2003 and Willcox et al., 2003). Also, the supplementation with such carotenoids reducing risk of age-related macular degeneration (AMD); cataract formation (Landrum and Bone, 2001) and alzheimer's disease (Barnham et al., 2004 and Passamonti et al., 2005). Therefore, in this study it is important to investigate the content of carotenoids and tocopherols as natural antioxidants in the fruits of seven date varieties. In addition, the effect of some technological treatments on these phytochemicals also was studied.

MATERIALS AND METHODS

Materials

Date Samples

Fruits of seven date varieties were used in this study. Fruits of soft (Samani and Zaghloul) and semi-dry (Saidy and Amri) dates were collected at Khalal stage from

Assiut University Farm. Manthour (semi-dry) and Sakkoti (dry) fruits were purchased from local market at Assiut and Aswan cities, respectively, in Egypt. Khudri (semi-dry) fruits were purchased from local market at Berlin, Germany (imported from Onaizah Dates Factory, Qassim, K.S.A.). Tow kilograms of the different date samples were collected at random during 2004 season. The samples were packaged in sealed polyethylene bags and stored at -20°C until analysis at Sugar Technology Institute, TU-Berlin, Germany..

Analytical Methods

Moisture was determined in according to Auda et al. (1976). Total soluble solids (TSS) of date juice and syrup were determined by a digital refractometer (Schmidt & Haensch, ATR W series).

Extraction of carotenoids and tocopherols

Date fruit samples were pitted and the free flesh was cut into small pieces and minced just before analysis. Representative samples (5 g) were taken, in triplicate, immediately after mincing and disintegrated in a crucible mortar in the presence of 1 g of quartz sand for extraction of carotenoids or tocopherols. The extraction was carried out by a previously described (Abushita *et al.*, 1997) method in which methanol was added first to catch water and make the transfer of lipophilic carotenoids and tocopherols to the less polar solvent more easier in the subsequent step. Brown colored conical flasks, round – bottom flasks and separatory funnels were used in the different analyses avoid light catalyzed degradation of photosensitive vitamins. A mixture of 60:20 (v/v) carbon tetrachloride – methanol containing (0.5%) butylated hydroxytolueune (BHT) was added, and the mixture was shaken for 15 min. The lower colored layer was separated in a separatory funnel and dried on anhydrous sodium sulphate (Na_2SO_4). The solvent was then evaporated under vacuum by Rotary Evaporator at maximum 40 °C. The residues were either redissolved in an aliquot of the HPLC eluent for carotenoids analyses or applied for saponification procedure for analysis of tocopherols.

Saponification of tocophorals

To the extracted pigment and tocopherols fraction, 5 ml of

saturated methanolic KOH, 0.5 g ascorbic acid, and 20 ml of methanol were added. The mixture was then saponified by refluxing for 30 min at boiling point of methanol. After cooling the flask, 15 ml of saline solution (15% Na Cl) salted water was added and the analogues of tocopherol were extracted twice with 40 ml of analytical grade n-hexane in a separatory funnel. The hexane fractions were collected, washed twice with distilled water, and dried on anhydrous Na_2SO_4. The solvent was evaporated under vacuum at 30 °C, and the residues were redissolved in 5 ml of HPLC-grade n-hexane for chromatographic analysis. Separation conditions and parameters for the HPLC determination of carotenoids and tocopherols were applied according to methods of Biacs and Daood (1994) and Speek *et al.* (1985), respectively. For the identification of peaks, the retention times and maximum absorption spectra of carotenoids and tocopherols were compared with those authentic standard materials, which were also used for quantification.

Statistical Analysis

Data of carotenoids and α-tocopherol concentration (on dry weight basis) of the studied date fruits and Khudri date products were subjected to statistically analysis according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Data of moisture content of the studied date cultivars are shown in Fig. 1. Moisture contents were 48.43% for Samani and 39.23% for Zaghloul (soft dates). The moisture of the semi-dry dates ranged from 9.93 to 55.33%. The semi-dry dates (at Khalal stage) contained high moisture levels except Khudri variety (over ripe), had the lowest value. Moisture content of Khudri fruits was less than the recorded average in semi-dry varieties, this may be due to a loss in moisture content during transportation and storage. These results are in line with those recorded by Ramadan (1995 and 1998).

The data given in Fig. 2 summarize the mean values of ß-carotene and lycopene of seven fruits date cultivars. Regarding ß - carotene content, the results showed that Saidy date cultivar contained the highest amount followed by Zaghloul fruits (3970.0 and 2767.5 µg/100g fresh

weight, respectively). While, Khudri fruits recorded the lowest ß-carotene value (80.0 µg/100g fresh weight). The different date varieties presented a lycopene content varied from 170.0 to 3695.0 µg/100g fresh weight for Manthour and Saidy fruits, respectively. It could be mentioned that there were detectable variations in ß-carotene and lycopene concentrations not only among all the studied date varieties but also within varieties of the same category (i.e., soft or semi-dry date group). It was clear that Saidy date flesh had the highest concentration of ß-carotene and lycopene followed by the soft date varieties; Zaghloul and Samani.

From the data in Fig. 3, it could be observed that the studied date varieties contained amounts of α-tocopherol ranging from 966.0 to 204.0 µg/100g fresh weight. Amri date fruits had the highest amount of α-tocopherol followed by Khudri, Manthour and Saidy date fruits, which are semi-dry dates.

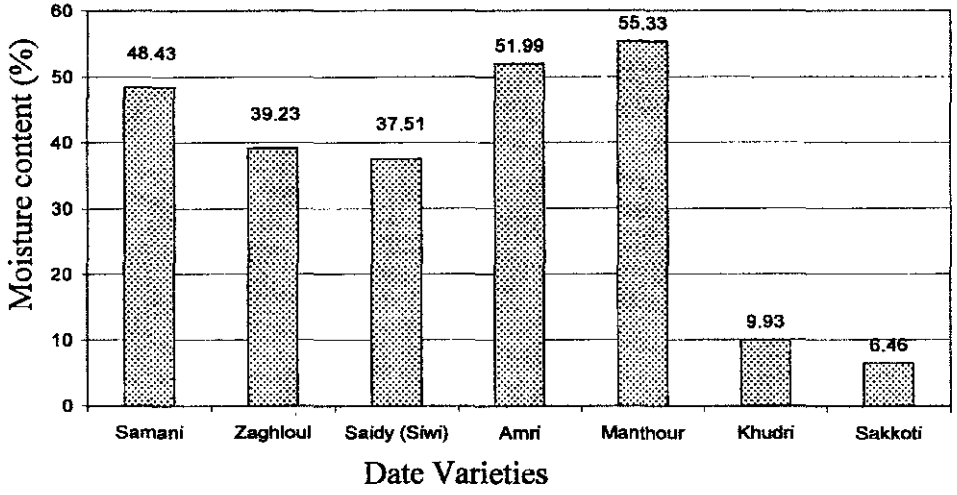

Fig.1. Moisture content of the studied date varieties.

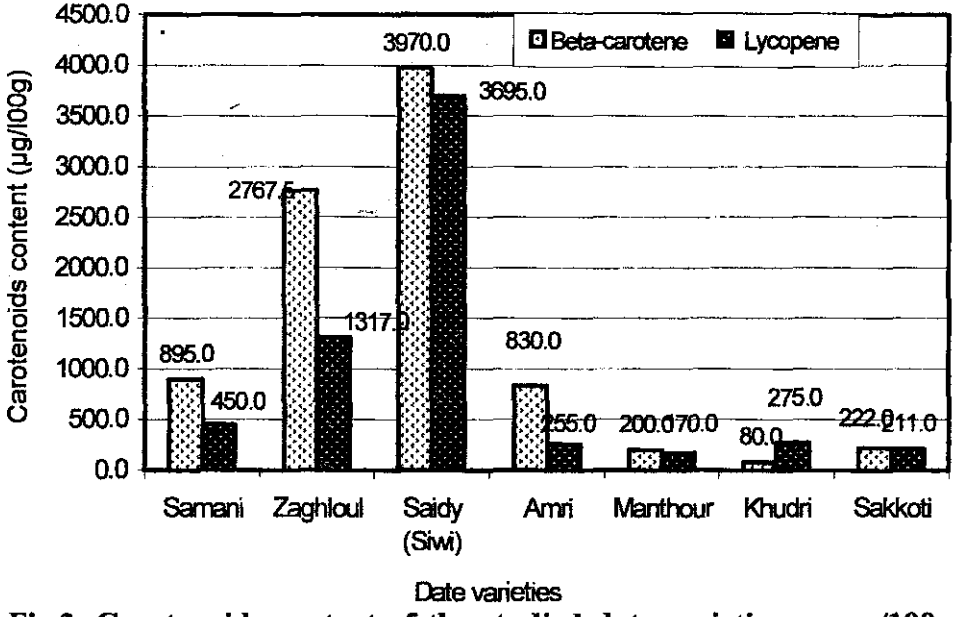

Fig.2. Carotenoids content of the studied date varieties as µg /100g wet weight.

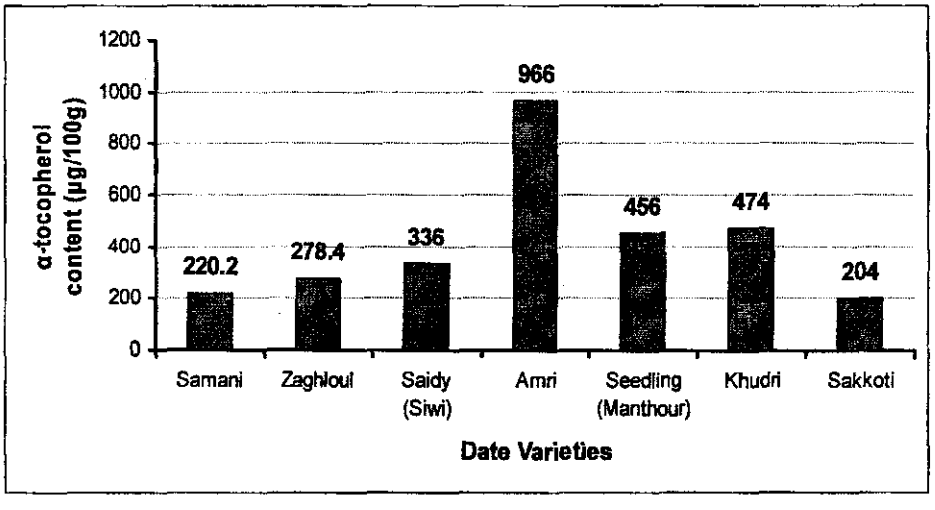

Fig.3. α-Tocopherol content of the studied date varieties as µg /100g wet weight.

The studied date fruits contained higher ß-carotene values than the most selected Australian fruits which were recorded by Mackerras (1995) such as apple, 5 µg; pear, 20 µg; banana, 65 µg; mandarin, 67 µg; peach and orange, 98 µg; plum, 147 µg; apricot and watermelon, 198 µg and mango fruits, 1550 µg/ 100g fresh weight. Kim *et al.* (2007) reported that seven raw Korean fruits had detectable quantities of α-tocopherol and ß-carotene. They recorded that α-tocopherol content of selected Korean fruits compared with the results of USDA (2006) was as follows: apple, 40-70 µg and 50 µg; grape, 60-100 µg and 190 µg; oriental melon, 50-70 µg and not detected; peach, 110-150 µg; plum, 90-100 µg and 260 µg and watermelon, 10-30 µg and 50 µg/100g fresh weight, respectively. Moreover, the same abovementioned studies recorded the contents of ß-carotene were 18.3-20.5 µg and 17.0 µg for apple; 20.3-26.8 µg and 39.0 µg for grape; 4.0- 8.5µg and not detected for oriental melon; 0.0-3.5 µg and 162.0 µg for peach; 31.5-54.4 µg and 190.0 µg for plum; and 424.5-547.9 µg and 303.0 µg/100g fresh weight for watermelon, respectively. While, lycopene was not detected in all the studied fruits except watermelon which had high concentration of lycopene ranged from 1640.0 to 2778.0 µg and 4532.0 µg/100g fresh weight according the same above studies, respectively.

Comparing the concentration order of ß-carotene, lycopene and α-tocopherol of the studied date fruits for fresh consumption with their concentrations calculated on dry weight basis, it was some marked variations due to initial moisture content. Statistically, there were highly significant differences in ß-carotene, lycopene and α-tocopherol contents (µg/ g dry weight) among all the studied date varieties except among Samani and Amri (soft and semi-dry varieties, respectively) in their ß-carotene contents (Table 1).

The recommended daily allowance of ß-carotene (converts to vitamin A in the body) and vitamin E (α-tocopherol) are 5000 IU (3000 µg) and 30 IU (20 mg), respectively for the adult human. These requirements are slightly increased for pregnant and lactating woman (Folk and

Wilkinson, 2006). From the present study results, 100g of fresh date · flesh (with, 80.0-3970.0 µg ß-carotene) will provide an adult human with 2.70 to 132.33% of his daily requirements. However, the same weight of date (with, 204.0-966.0 µg α-tocopherol) will give only 1.02 to 4.83% of his daily requirements.

Moisture contents of Khudri raw juice, sterilized juice, syrup and by- product were 83.40, 83.59, 19.57 and 67.50%, respectively (Fig. 4).

ß-carotene and lycopene contents of different Khudri date products are shown in Fig. 5.

ß-carotene and lycopene ranged from 10 to 20 µg and from 40 to 65 µg/ 100g fresh weight date products, respectively. While, both components; ß-carotene and lycopene were not detected in the residue (by-product). Moreover, raw juice, sterilized juice and by-product had α-tocopherol values as 198, 168 and 324 µg/ 100g fresh weight, respectively (Fig. 6).

To avoid the effect of water evaporation and concentration of solids taking place during thermal processing on the quantification, the estimated values were weight-based (per g of dry matter).

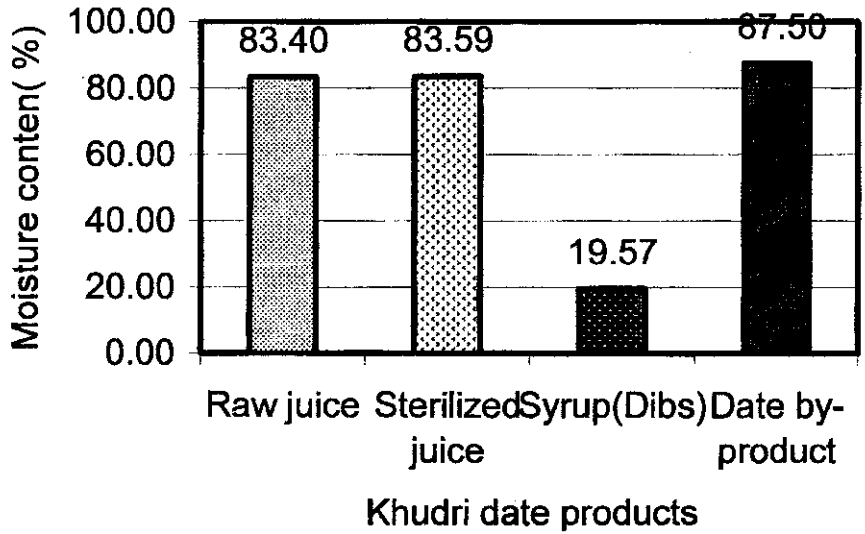

Fig.4. Moisture content of different date products of Khudri fruits.

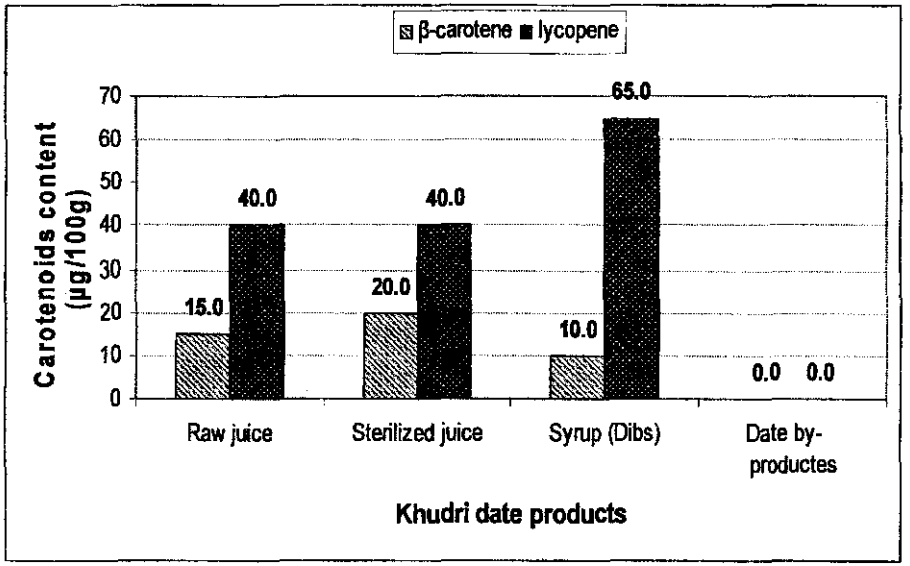

Fig.5. Carotenoids content of different date products of Khudri fruits µg / 100g wet weight.

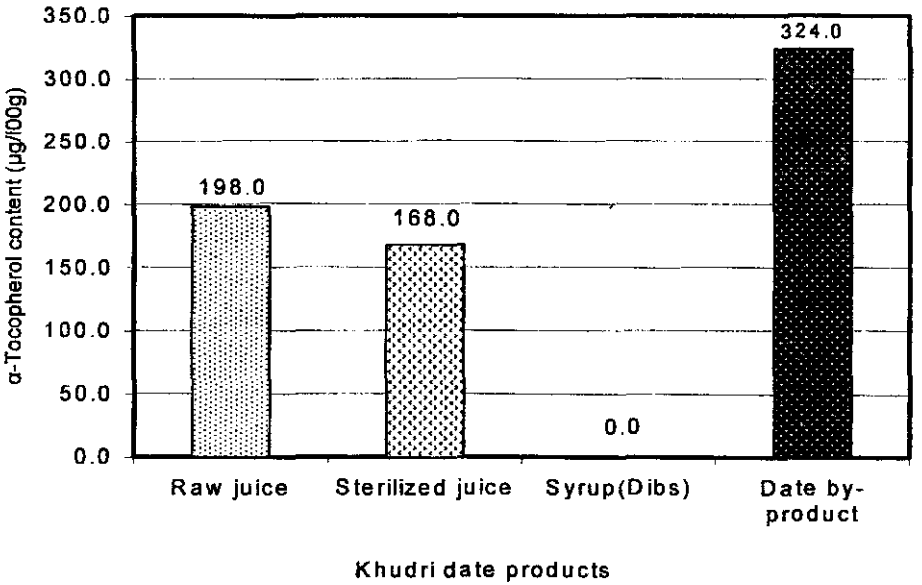

Fig. 6. α-Tocopherol content of different date products of Khudri fruits as µg /100g wet weight.

With regard to the processing effect of the studied antioxidant components, it was observed that the heat treatment during the preparation of raw date juice occurred an increase in ß-carotene (1.75 %) and α-tocopherol (126.65 %) levels, compared with the raw fruits, while, lycopene content was decreased. The sterilized date juice recorded the highest ß-carotene level (1.22 µg/ g dry weight). Boileau et al. (1999) reported that some carotenoids such as α- and ß-carotene and lycopene survive heat treatment. Southon (2000) found that the bioavailability of carotenoids increased fivefold in the processed products as a consequence of moderate heating or enzymatic disruption of vegetable cell structure.

The difference between Khudri fruits and its products in their ß-carotene contents proved to be highly significant except in-between the fruits and the raw juice (Table 4). Also, it could be observed that there were high significant differences in the date fruits and its products for lycopene and α-tocopherol contents except within the raw and sterilized juices. Besides, the processing of date syrup (centrifugation, heating of raw juice and evaporation under vacum) decreased both ß-carotene and lycopene in the syrup by 86.24 and 66.46%, respectively compared with the raw juice, while, α-tocopherol was not detected. The date by-product had high level of α-tocopherol (5.26 µg/ g dry weight). It is well known that naturally occurring antioxidants could be significantly lost as affected by processing and storage (Kaur and Kapoor, 2001). Jonsson (1991) reported that thermal treatments are believed to be the main cause of the depletion in natural antioxidants in food. Large processing is believed to be responsible for losses in natural antioxidant activity (Miller et al., 1995). But recent research has now established that food processing has also some positive roles which improve the quality health properties of the processed food. This is mainly attributed to the increased bioavailability of some antioxidants such as ß-carotene (Talcott et al., 2000). They found that the antioxidant levels increased immediately after heat processing by 34.3 % in canned carrot. Generally, thermal processing caused a decrease in α-tocopherol and an increase in carotenes.

Table 1. Statistical analysis of ß-carotene, lycopene and α-tocopherol contents of different date fruits (µg/g dry weight).

Date varieties	Carotenoids		α-tocopherol
	ß -carotene	Lycopene	
Samani	$17.36^{c} \pm 0.16$	$08.76^{c} \pm 0.18$	$04.27^{f} \pm 0.07$
Zaghloul	$45.57^{b} \pm 0.19$	$21.76^{b} \pm 0.16$	$04.58^{e} \pm 0.10$
Saidy (Siwi)	$63.53^{a} \pm 0.53$	$59.13^{a} \pm 0.13$	$05.78^{c} \pm 0.08$
Amri	$17.29^{c} \pm 0.10$	$05.31^{d} \pm 0.10$	$20.12^{a} \pm 0.12$
Seedling (Manthoui	$04.48^{d} \pm 0.08$	$03.81^{e} \pm 0.10$	$10.21^{b} \pm 0.21$
Khudri	$00.89^{f} \pm 0.02$	$03.05^{f} \pm 0.05$	$05.26^{d} \pm 0.06$
Sakkoti	$02.37^{e} \pm 0.10$	$02.26^{g} \pm 0.06$	$02.19^{g} \pm 0.09$
F-test 0.05	**	**	**

Results are means ± SD (n =3). Values of the same column followed by the same letter, are not statistically different ($p \leq 0.05$).
** : F value is high significant.

Table 2. Statistical analysis of ß-carotene, lycopene and α-tocopherol contents of Khudri fruits and its products (µg/g dry weight)

Date products	Carotenoids		α-tocopherol
	ß -carotene	lycopen	
Khudri fruits	$0.89^{b} \pm 0.02$	$3.05^{a} \pm 0.05$	$05.26^{d} \pm 0.06$
Raw juice	$0.91^{b} \pm 0.04$	$2.41^{b} \pm 0.11$	$11.93^{b} \pm 0.13$
Sterilized juice	$1.22^{a} \pm 0.10$	$2.44^{b} \pm 0.11$	$10.24^{c} \pm 0.12$
Syrup (Dibs)	$0.12^{c} \pm 0.02$	$0.81^{c} \pm 0.03$	Nd
Date by-product	Nd	Nd	$25.92^{a} \pm 0.12$
F-test 0.05	**	**	**

Nd= Not detected
Results are means ± SD (n =3). Values of the same column followed by the same letter are not statistically different ($p \leq 0.05$).
** : F value is high significant.

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تأثير الأصناف و التصنيع علي محتوي ثمار البلح من الكاروتينويدات والألفا توكوفيرول

بلبل رمضان رمضان
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تم دراسة محتوي ثمار سبعة أصناف من التمور (طرية ، نصف جافة ، جافة) من مضادات الأكسدة النباتية: البيتا كاروتين ، الليكوبين والألفا توكوفيرول. وقد أظهرت النتائج أن ثمار بلح الصنف الصعيدي (السيوي) تحتوي كمية من البيتا كاروتين (٠,٣٩٧٠ ميكروجرام/ ١٠٠جم وزن طازج) يليها ثمار البلح الزغلول (٠,٢٧٦٧ ميكروجرام/ ١٠٠جم وزن طازج) ، بينما سجلت ثمار البلح الخضري أقل مقدار (٠,٨٠ ميكروجرام/ ١٠٠جم وزن طازج). وقد احتوت ثمار أصناف البلح تحت الدراسة مقداراً من الليكوبين تراوح بين ٠,١٧٠ إلي ٠,٣٦٩٥ ميكروجرام/ ١٠٠جم وزن طازج من ثمار البلح البذري والبلح الصعيدي علي التوالي. كما احتوت ثمار البلح تحت الدراسة مقداراً من الألفا توكوفيرول يتراوح بين ٠,٢٠٤ إلي ٠,٩٦٦ ميكروجرام/ ١٠٠جم وزن طازج. وعموماً فقد أتضح أن هناك فروقا معنوية بين جميع ثمار البلح المدروسة في محتواها من كل من البيتا كاروتين ، الليكوبين والألفا

توكوفيرول (ميكروجرام/جم علي أساس الوزن الجاف) فيما عدا بين البلح السماني والبلح العمري (صنف طري ، ونصف جاف ، علي التوالي) في محتواها من البيتا كاروتين.

ومن النتائج المتحصل عليها يمكن القول بأن كل ١٠٠جم تؤكل من ثمار البلح الطازج تحت الدراسة يمكن أن تمد الشخص البالغ بمقدار من البيتا كاروتين يتراوح بين ١,٧٠ إلي ١٣٢,٣٣ % من مقدار الاحتياجات اليومية له منها، كما أن نفس الوزن يمكن أن يمده بين ١,٠٢ إلي ٤,٨٣ % من الاحتياجات اليومية له من الألفا توكوفيرول.

كما تم دراسة تأثير المعاملات التكنولوجية علي محتوي منتجات بلح الخضري من المواد المضادة للأكسدة النباتية تحت الدراسة، حيث لوحظ أن المعاملة الحرارية خلال إعداد عصائر البلح الخضري قد أدت إلي زيادة مستوي البيتا كاروتين بمقدار ١,٧٥ % والألفا توكوفيرول بمقدار ١٢٦,٦٥ % ، مقارنة بمستواها في الثمار الخام ، بينما انخفض محتواها من الليكوبين بمقدار ٢١,٠٧ %- بالإضافة إلي أن عمليات إعداد عسل البلح (الدبس) أدت إلي زيادة النقص في محتوي العسل الناتج من كل من البيتا كاروتين والليكوبين مقارنة بالعصير الطازج (الخام) ، في حين أنه لم يحتوى على أي كمية من الألفا توكوفيرول. وقد احتوي اللب المتخلف كناتج ثانوي في صناعة عسل البلح علي نسبة عالية من الألفا توكوفيرول (٥,٢٦ ميكروجرام/ جم وزن جاف).