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**AMINO ACID PROFILE, VITAMIN AND REDUCING SUGAR COMPOSITIONS OF
ETHANOL FRUIT-EXTRACT OF *Phoenix dactylifera L* (DATE FRUIT) SOLD IN
ABAKALIKI, EBONYI STATE, NIGERIA**

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ABSTRACT

Amino acid profile, vitamin and reducing sugar compositions of *Phoenix dactylifera* fruit (Dates fruit) sold in Abakaliki, Ebonyi State, Nigeria were evaluated. Amino acid profile was carried out using amino acid analyzer. Vitamin and sugar analyses on the ethanol fruit-extract of *Phoenix dactylifera L* were carried out using standard procedure outlined by Association of Official Analytical Chemists (A.O.A.C). The result of amino acid profile obtained showed that cysteine was not detected in the sample and nineteen (19) other amino acids were detected with proline (0.89%) and tryptophan (0.78%) as the major amino acids. Trimethylsone (<0.1%) was also detected in the sample. Result of vitamin composition obtained revealed Ascorbic acid

(Vitamin C) as the vitamin with the highest value of 65.17 ± 0.080 mg/100g and folic acid (vitamin B₉) with the least value of 0.065 ± 0.040 mg/100g. Sugar analysis of the extract showed that sucrose has the highest value of 8.64 ± 0.5 mg/100g followed by fructose with a value of 4.16 ± 0.25 mg/100g and glucose has the lowest value of 0.98 ± 0.15 mg/100g. This study indicates that date fruit is a good source of essential amino acids, vitamin C and energy and can contribute immensely in both human and animal nutrition and health.

Keywords: *Phoenix dactylifera* Fruits, Vitamin, Amino acid profile, Reducing sugar and Ethanol Fruit- Extract.

INTRODUCTION

Phoenix dactylifera (date fruit) are sweet and delicious fruits from the tropical oasis. The date fruit is a "drupe", in which its outer fleshy part (exocarp and mesocarp) surrounds a shell (the pit or stone) of hardened endocarp with a seed inside [1]. The fruits grow on the palm tree belonging to the family of *Arecaceae*, in the genus *Phoenix*, and scientifically named as *Phoenix dactylifera* [1]. Date fruit originate from the land on the banks of Nile and Euphrates Rivers of ancient Egypt and Mesopotamia [1]. Date palm is now grown extensively for its edible fruits under warmer climates across all the continents. In Nigeria it is called *Dabino* in Hausa and is commonly used by Nigerian Muslims to break their Ramadan fast [1]. Dates palm can take 4 to 8 years after planting before they will bear fruit, and produce viable yields for commercial harvest between 7 to 10 years. Mature date palms can

produce 68 to 176 kilograms (150 to 300lb) of dates per harvest season, although they do not all ripe at the same time so several harvests are required in order to get fruit of marketable quantity. The bunches of dates must be thinned and bagged or covered before ripening so that the remaining fruits grow larger and are protected from weather and pests such as birds.

There are many varieties of dates palm cultivar. 'Amir Hajj', 'Saidy', 'Khadrawy' and 'Medjool' are some of the important varieties popular for their rich taste, flavour, and superior quality. The most common consume varieties of date fruit in Nigeria are Dagalla, Dan-Mali and Fari [2]. Dates fruits are considered a complete diet and a very important item of food. With plenty of vitamins and minerals, dates have 25% more potassium than bananas while being free from fat, sodium and cholesterol. Dates fruits

play an important role in the diet and treatment of obesity and are the most important sources of energy and food in date producing and non date producing areas [3]. In Nigeria, particularly in the northern part, date fruit has become a major source of nutrition and energy source among Muslims and even the non-muslim communities. For Muslims, it is a customary religious meal for breaking day long fast during the holy month of Ramadan [2]. Date fruits are the staple food of the Middle East and very popular in Islamic countries. Dates fruits are considered a complete diet and a very important item of food. The date fruit is consumed fresh and also used in the preparation of syrup, jams, table jellies, paste and dried date [4].

Vitamins are critical organic molecules necessary for life. Despite their vital importance, they cannot be synthesized by the body and therefore must be acquired through diet, whether they are obtained from food or through dietary supplements [5]. It is more efficient to obtain vitamins in food in trace amounts than to develop the numerous enzymes necessary to synthesize vitamins. In addition, vitamins must be molecularly modified after ingestion before they can be used in the body. Vitamins can act as coenzymes, signaling molecules, antioxidants, and hormones, as well as

serving various other functions [5]. Vitamin deficiency can lead to conditions such as: scurvy from lack of Vitamin C, and eye damage from lack of Vitamin A and others [5]. In fact, many activated carriers in metabolism are derived from vitamins, including electron carriers important for redox reactions, such as electron transport (FADH₂, for example), and carbon dioxide transfer (biotin). Because of the critical roles vitamins play in the functioning of the human body, they are an essential topic of study despite the low vitamin requirements of most animals.

Amino acids is defined as biologically important organic compounds containing amino (-NH₂) and carboxyl (COOH) functional groups, usually along with a side-chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, though other elements are found in the side-chains of certain amino acids. Amino acids comprise the second-largest component (water is the largest) of human muscles, cells and other tissues. Outside proteins, amino acids perform critical roles in processes such as neurotransmitter transport and biosynthesis. Amino acids can be placed in the category of either essential or non-essential.

Reducing sugar on the other hand is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or free ketone group. All monosaccharides are reducing sugars, along with some disaccharides, oligosaccharides, and polysaccharides. The monosaccharides can be divided into two groups: the aldoses, which have an aldehyde group, and the ketoses, which have a ketone group. Ketoses must first tautomerize to aldoses before they can act as reducing sugars. The common dietary monosaccharides such as galactose, sucrose, glucose and fructose are all reducing sugars. The nature and concentration of sugars and organic acids in fruits have been of interest because of their important influence on the organoleptic properties and their important roles in maintaining fruit quality and determining nutritive value [2].

Though there are several documented studies around the world on nutritional compositions of different varieties of date fruits, therefore, there is a need to evaluate the amino acid profile, vitamins and reducing sugar compositions of date fruits commonly sold in Abakaliki, Ebonyi State, Nigeria to enable its utilization beyond the tradition use for

breaking of fast during Ramadan and more especially this era malnutrition is besieging millions of people in Nigeria and other developing countries.

MATERIALS AND METHODS

Materials

Collection and Preparation of *Phoenix dactylifera* Fruits

The dried fruits of *Phoenix dactylifera* were purchased from Hausa Quarter Abakaliki, Ebonyi State and were identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. A part of the plant was also kept in the herbarium for reference purposes. The fruit of *Phoenix dactylifera* were dried at room temperature and ground to powdered form using electrical grinding machine. Exactly 200g of grinded *Phoenix dactylifera* fruits were soaked in ethanol and were allowed to stand for 48 hrs. The mixtures were filtered using clean sieve cloth and the filtrate evaporated leaving the extract behind. The extract was kept in a dried clean container and stored in a refrigerator.



Figure 1: *Phoenix dactylifera* fruits

Chemical and Reagents: The chemicals and reagents used for the analyses were of analytical grades.

Methods: Amino acid profile of the ethanol fruit extract of *Phoenix dactylifera L* was carried out using amino acid analyzer while vitamin and sugar analyses were carried out using standard procedure outlined by Association of Official Analytical Chemists, [6], as shown below:

Determination of Vitamin A: Exactly 1g of the sample was macerated in 20ml of

$$\text{Vitamin A (mg/L)} = \frac{\text{Absorbance of sample} \times \text{Dilution factor} \times \text{Gradient factor}}{\text{Weight of sample}}$$

Determination of Vitamin E: Exactly 1g of the sample was macerated in 20ml of petroleum ether for 10mins and allowed to stand for 1hr with intermittent shaking at every 10mins. It was centrifuged for 5mins and 3ml was transferred into triplicate tubes,

petroleum ether and allowed to stand for 1hr. It was shaken gently at 10mins interval. It was centrifuged for 5mins and 3ml was transferred into triplicate tubes; in a water bath and it was evaporated to dryness. About 0.2ml of acetic anhydride/chloroform was added to the residue. 2ml of 50% TCA in chloroform was added to the mixture. The absorbance was read at 620nm.

evaporated to dryness and 2mls of ethanol was added. After shaking, 1ml of 0.2% ferric chloride was added to ethanol and mixed. 1ml of ethanol was added again to make it 5ml. Absorbance was read at 520nm.

$$\text{Vitamin E (mg/L)} = \frac{\text{Absorbance of sample X Dilution factor X Gradient factor}}{\text{Weight of sample}}$$

Determination of Vitamin D: Exactly 1g of the sample was macerated in 20ml of petroleum ether for 10mins and allowed to stand for 1hr with intermittent shaking at every 10mins. It was centrifuged for 5mins, and then 3ml was transferred into triplicate tubes. After evaporation to dryness, 2ml of alcohol potassium hydroxide was added and

boiled for 30mins. 0.5ml of 0.1% pyrogallol was added in ethanol and 4 drops of 10% aluminum chloride was also added to ethanol and heated in water bath for 4mins. After cooling, 4.5ml of ethanol was added and mixed. The absorbance was read at 470nm and 500nm.

$$\text{Vitamin E (mg/L)} = \frac{\text{Absorbance of sample X Dilution factor X Gradient factor}}{\text{Weight of sample}}$$

Determination of Vitamin K: Exactly 1g of the sample was macerated in 20ml of petroleum ether for 10mins and allowed to stand for 1hr with intermittent shaking at every 10mins. It was centrifuged for 5mins and 3ml was transferred into triplicate tubes. After evaporation to dryness, 2ml of water

and 1ml of 0.04% 2,4-dinitrophenyl hydrazine was added to 1:5 Hydrochloric acid and boiled in water bath for 45mins. After cooling, 10ml of 1:30 ammonium hydroxide was also added and mixed. Absorbance was taken at 635nm against the blank.

$$\text{Vitamin K (mg/L)} = \frac{\text{Absorbance of sample X Dilution factor X Gradient factor}}{\text{Weight of sample}}$$

Determination of Vitamin B₁: Exactly 1g of the sample was macerated in 20ml of Water for 10mins. It was centrifuged for 5mins and 1ml was transferred into triplicate tubes. 1ml of a reagent comprised of one part of 1% potassium ferricyanide and a part of 10% sodium hydroxide was added and mixed. After 1min, 7.5ml of isobutyl alcohol was added and was shaken for 2mins by moving the test tube up and down. It was given some time to separate the isobutyl layer was collected and centrifuged for 5min.

absorbance was taken at 367nm against isobutyl alcohol as blank.

Determination of Vitamin B₂: Exactly 1g of the sample was macerated in 20ml of water for 10mins. After centrifuging for 5mins, 1.5ml of the supernatant was transferred into triplicate tubes. 6.5ml of water and 2ml of deidenigen reagent was also added, mixed and allowed to stand for 15mins. The absorbance was read at 525nm.

Determination of Vitamin B₆: Exactly 1g of the sample was macerated with 20ml of

water for 10mins. It was centrifuged for 5mins. 1ml of the supernatant was transferred into triplicate tubes and 2ml of water was added. 0.4ml of 50% of sodium acetate, 0.1ml of deoxidized reagent and 0.2ml of 5.5% sodium carbonate was mixed and added. The absorbance was then read 540nm.

Determination of Vitamin C: Exactly 0.5g of the sample was macerated in 20ml of 0.4% of oxalic acid for 10mins and centrifuged for 5mins. 1ml of the supernatant was transferred into triplicate tubes. 9ml of 2, 6-dichlorophenyl indophenol (12 mg/L) was added and mixed. Absorbance was read at 520nm after 15sec and 30sec.

Determination of Vitamin B₃: Exactly 20ml of 1N H₂SO₄ was added to 1g of the sample and was shaken for 30mins. 5ml of the filtrate was transferred to another test tube and 2.5ml of potassium cyanide (1g/100ml) was added. It was finally acidified with 5ml of 0.02% NH₂SO₄. The absorbance was read at 470nm.

Determination of Fructose: Exactly 0.1ml of the sample was weighed and macerated in 0.9ml of distilled water. 1ml of the reagent A (RA = 50mg resorcinol dissolved in 50ml of ethanol) was added together with 1ml of the reagent B (R_B = 50ml concentrated HCl + 10 mls of distilled H₂O). It was then boiled for 8mins and allowed to cool at room

temperature. The absorbance was read at 530nm.

Determination of Sucrose: Exactly 1ml of sample plus 9mls of H₂O in solution was made. 1ml of the mixture were taken and added to 5ml of 2% Orsinol. It was boiled for 12mins and allowed to cool and made up to 25mls with H₂O. Absorbance was read at 620nm.

Determination of Glucose: Exactly 1ml of the sample were taken and macerated in 9ml of H₂O. 1ml of alkaline solution was added which turned the mixture taint blue. It was boiled for 5mins and allowed to cool under room temperature. Finally, 1ml of phosphomolybdc acid was added and shaken gently. Absorbance was read at 420nm.

Determination of Amino Acid Profile: Amino acid profile was determined based on the method described by [7]. Technicon Sequential Multi-sample amino acid Analyzer (TSM-1 Technicon instrument Basingstoke, Uk) using Nor-leucine as an internal standard. About 5-10ml of sample was dispensed into the cartridge of the analyzer. The TSM analyzer is designed in such a way as to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The analysis lasted for 76mins.

RESULTS

Results of Vitamin, Amino acid and reducing Sugar Analyses of *Phoenix dalactylifera* Fruits

The results of vitamin composition of *Phoenix dalactylifera* fruits revealed that Vitamin C and Vitamin A are the major vitamin constituents while others are in a minute amount as shown in Figure 2. The result obtained from the analysis of amino acid profile of *Phoenix dactyfera* fruit revealed that proline has the highest value, followed by typtophan, threonine, aspartic

acid, argenine, alanine, valine, serine, asparagine, phenylamine, leucine, methionine, tyrosine, histidine, isoleucine, glytamic acid, glycine and glutamine. Trimethysone was also detected in the sample while cysteine was not detected as shown in Figure 3. The result of reducing sugar analysis revealed that glucose has the highest value followed by fructose and sucrose which has the least value as shown in Figure 4.

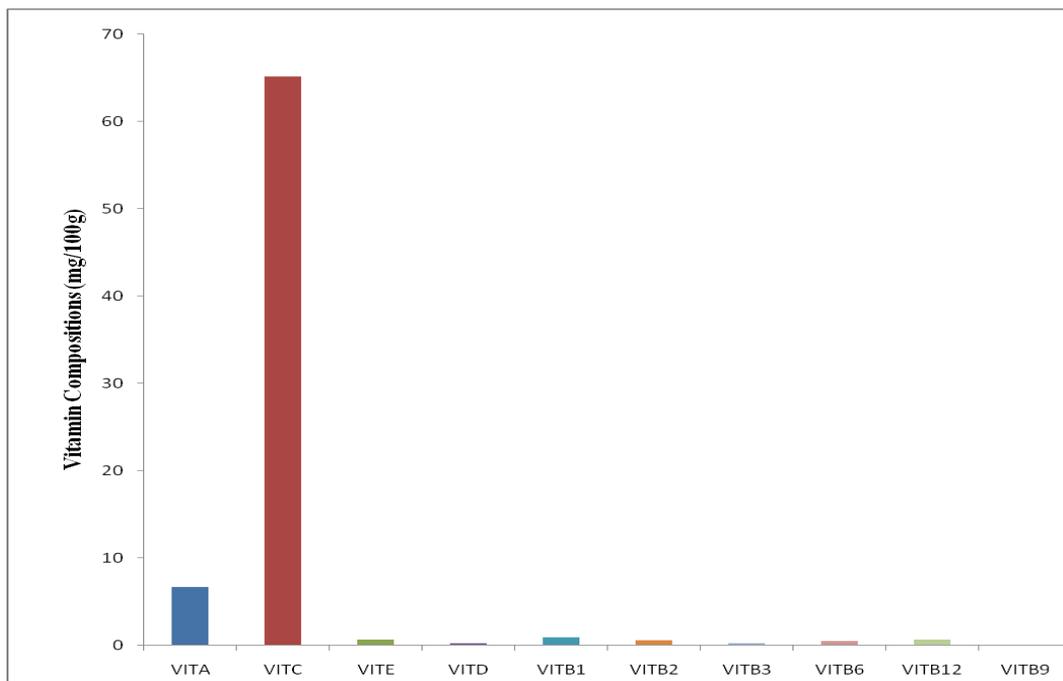


Figure 2: Vitamin Composition of *Phoenix dactylifera* Fruits

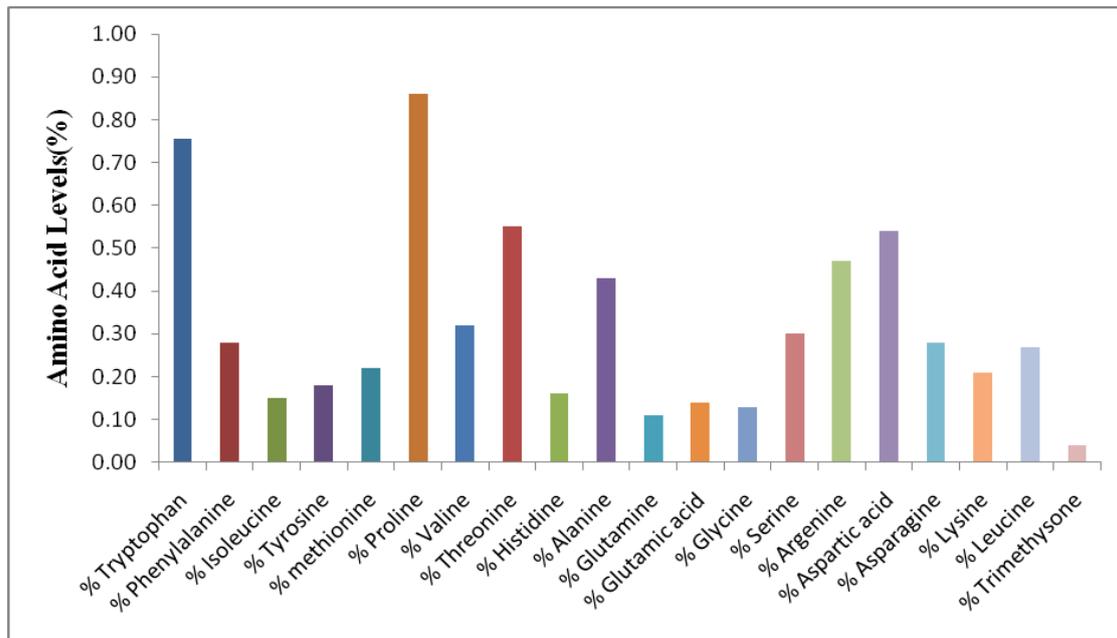


Figure 3: Amino acid Profile of *Phoenix dactyfera* Fruits

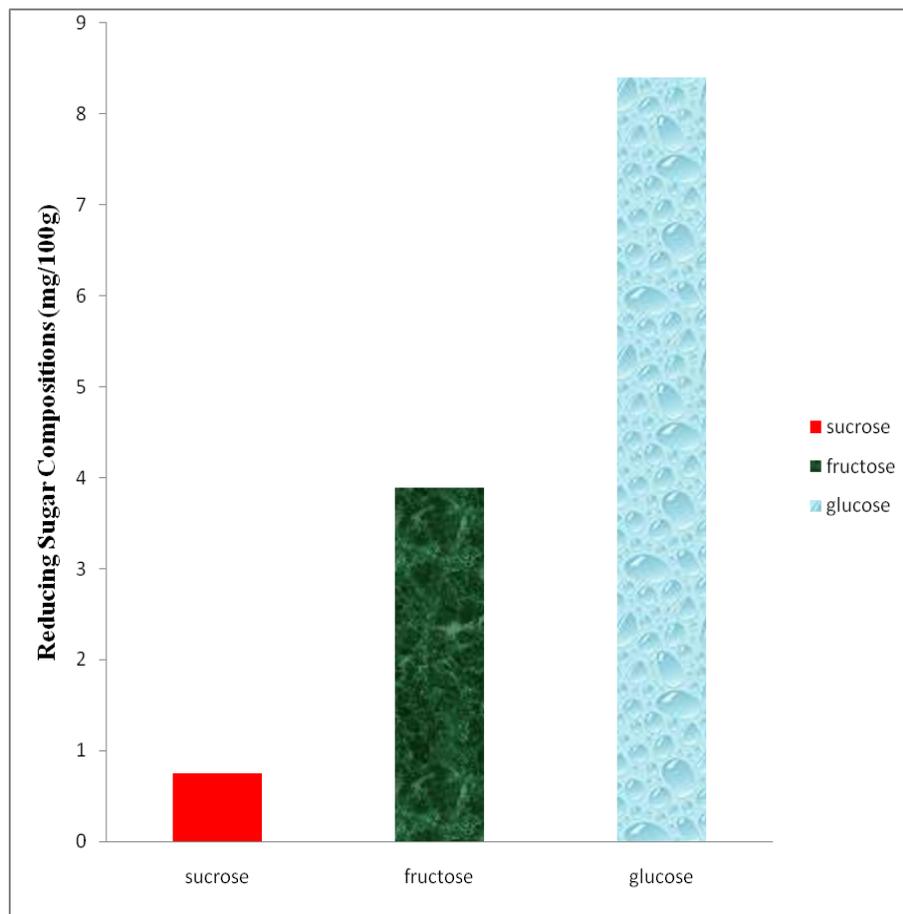


Figure 4: Reducing Sugar Compositions of *Phoenix dactyfera* Fruits

DISCUSSION

The result showed that date fruits sold in Abakaliki contain vitamins such as Vitamin C, Vitamin A, Vitamin B₁, Vitamin B₂, Vitamin E, Vitamin B₁₂ and Vitamin B₆. The result of this study revealed that *Phoenix dactylifera* fruits are a good source of Vitamin C and Vitamin A (Figure 2) which is in correlation with the finding of Aja *et al.* (2016), [8], that reported that *Pterocarpus santalinoides* leaves are good source of vitamin C and fat soluble vitamins. Offor *et al.* (2014), [9], also reported that Fresh Leaves of *Cucurbital moschata* and *Amaranthus hybridus* are good source of vitamins. USDA (2015), also reported the vitamin composition of *Malus domestica* (Apple fruit, a dried fruit similar to date) as Vitamin B₉ (3ug, 1%), Vitamin B₃ (0.091mg, 1%), (0.061mg, 1%), Vitamin B₅ (0.061mg, 1%), Vitamin B₆ (0.041mg, 3%), Vitamin B₂ (0.026mg, 2%), Vitamin B₁ (0.017mg, 1%), Vitamin A (54IU, 2%), Vitamin C (4.6mg, 8%), Vitamin E (0.18mg, 1%) and Vitamin K (2.2ug, 2%). Dates contain small amounts of ascorbic acid, thiamine, riboflavin and nicotinic acid, and Elleuch *et al.* (2008), [10], had reported that date fruits have strong antioxidant, anticancer and antiviral activities. The recommended nutrient intakes for Vitamin C according to

Food and Agricultural Organization/ World Health Organization expert consultation on human vitamin requirement for infants is 35mg/day, adult 45mg/day and adolescent (10-18 years), pregnant mother and lactating mothers are 40 mg/day, 55mg/day and 70mg/day respectively [11]. The study revealed vitamin C content in *Phoenix dactylifera* fruit to be 65.1703±0.08 mg/100g which means that *Phoenix dactylifera* fruit is a good source of ascorbic acid can be used to cure scurvy and protect against scurvy from unborn children in the womb. Uba *et al.* (2015), [2], reported that the minimum amount of Vitamin C needed to cure scurvy in men to be less than 10 mg/day. Vitamin A helps vision by keeping cells which are used for transduction of light into nerve signals healthy and the analysis of Vitamin A in *Phoenix dactylifera* fruit showed that it's a good source of Vitamin A.

The result obtained also revealed that all nineteen (19) out twenty amino acids were detected in *Phoenix dactylifera* fruits except cysteine. Proline was observed to be the major amino acid followed by tryptophan. Other essential amino acids detected in the seeds includes: lysine, isoleucine, leucine, methionine, threonine, valine and phenylalanine. This result agrees with the result of Uba *et al.* (2015), [2], that reported

that dates fruits from three different varieties contained a high concentration of aspartic acid, proline and alanine. The amino acids found in this study were not in agreement with amino acids composition of three Omani date cultivars at the tamar stage as reported by [12]. Observed differences may be due to cultivation condition, soil type or method used for analysis [13]. Eman and Reman (2015), [14], revealed the presence of 17 different amino acids, including glutamic acid that was foremost in the seeds, representing 17-18% of total amino acids. The result showed that Trimethylsone was also detected to be present in the fruit. Amino acids are important biomolecules that both serve as building blocks of proteins and are intermediates in various metabolic pathways. They serve as precursors for synthesis of a wide range of biologically important substances including nucleotides, peptide hormones, and neurotransmitters. Moreover, amino acids play important roles in cell signaling and act as regulators of gene expression and protein phosphorylation cascade [15], nutrient transport and metabolism in animal cells [16], and innate and cell-mediated immune responses.

The results as presented in Figure 4 showed that sugars in date fruits consist of a mixture of fructose ($C_6H_{12}O_6$), glucose ($C_6H_{12}O_6$) and

sucrose ($C_{12}H_{22}O_{11}$). Fructose and glucose were identified as the principal sugars and sucrose was detected in negligible concentrations. It could be due to the fact that it may be converted to sugars such as glucose and fructose [17]. Glucose, fructose and sucrose are easy to digest in human cells and useful for getting the energy for metabolic processes. Arabs, who eat them on a regular basis, show an extremely low incidence rate of cancer and heart disease [18]. Sugar accumulation, especially the concentration of a high level of fructose and glucose is a very important physiological process that determines the dessert fruit quality. Due to the fact that fructose is about two-fold sweeter than glucose, the fruit sweetness contributes well with the fructose concentration [19]. The number of sugars identified and their quantities in this study were in agreement with the results of Al-Farsi *et al.* (2005), [12], for some Omani date cultivars. The result is also in correlation with the results of, [20], which reported that the total sugar content in 12 United Arab Emirates date cultivars varied from 44.3 to 64.1 g/100 g and fructose/glucose ratio was about 1 [20].

CONCLUSION

The result revealed that ethanol fruit-extract of *Phoenix dactylifera* is a good source of

vitamin C and contains suitable amounts of essential amino acids and trimethylsone and also good source of sugar. These may inform the use of this fruit in ethno-medicine and energy source. However, the need to explore *Phoenix dactylifera* fruits should be supported and encouraged due to its immense benefit and importance to medicine, traditional, pharmaceuticals and dietary industries since it contains bioactive compounds.

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