Study of Nutritional Contents of PrunusAmygdalusBatsch Seeds

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ABSTRACT

Background: *Prunus amygdalus* Batsch (Rosaceae) is one of the major tree crops of the world.Inaddition to its nutritional value, almond also contains a number of phytochemicals that are responsible for a variety of bioactivities, among which antioxidant activity which has been frequently studied.Noprevious studies were done on Umm alfahm cultivar, this encouraged the authors to perform the present study aiming to throw light on the nutritional profile of title plant.

Material and methods: Proximate analysis of the kernel *Prunus amygdalus* B. includes the determination of moisture content, total ash, total protein, total fats, total carbohydrates and crude fibers, study of vitamin E content by using HPLC, determination of the mineral content according to Association of Official Analytical Chemists(A.A.O.C) and determination of amino acid contents in kernels.

Results: Almond were showed a total fat (52.083%) the protein constitute (22.83%), carbohydrate (16.9%) adequate low moisture content (4.37%) low ash content (3.82%), the content of vitamin E in almond (77.67 mg/100gm), the mineral contents of almond were resulted Calcium (0.23%) Phosphorus (0.53%), Potassium 6656 mg/kg, Magnesium 2864 mg/kg, Sodium 1072.5 mg/kg, Iron119.9 mg/kg, Zinc 52.25 mg/kg, Selenium 68.71 mg/kg and Copper 13.55 mg/kg. The identified amino acids amounted to (40.2%) calculated on dry basis, total non-essential amino acids percentage (70%) was higher than the percentage of the essential amino acids (30%). Glutamic acid (5.02%) was the major non-essential amino acid identified, whereas Cysteine (0.37%) was the minor component, concerning the identified essential amino acids, Leucine was the major one (1.38%), while methionine (0.16%) was the minor component.

Conclusion: From the present study, it could be concluded that the kernels of *Prunus amygdalus* Batsch"Umm alfahm" cultivated in Egypt are good for health and should readily be incorporated into diet.

Keywords: nutritional, *Prunus amygdalus*, almond, Umm alfahm, amino acid

INTRODUCTION

Almond area in Egypt occupies about (15944) feddans yielding mainly (22292 tons/year) according to **Egyptian Ministry** of Agriculture statistics^[1], most of this acreage is located in north Sinai, west Nubaria, Matrouh, south Sinai and Alexandria. The almond Prunus amygdalus, syn. Prunus dulcis, or Amygdalus communis is a small deciduous tree belonging to the subfamily Prunoideae of the family Rosaceae. Almond is one of the major tree crops of the world, almonds are one of the oldest commercial nut crops of the world; from the middle and west Asia, it has diffused to other regions and continents which include the Middle East,

China, the Mediterranean region and America.^[2]

Almond seeds, whole nuts or with skin peeled, are consumed as snacks or used as ingredients for processed foods such as various bakeries. confectioneries. and chocolates, whereas the inedible counterparts, including hull, shell, and skin are discarded or used as fuel material or livestock feed.^[3] Almond fruit consists of three or correctly four portions: kernel or meat, middle shell, outer green shell cover or almond hull and a thin leathery layer known as brown skin of meat or seedcoat. The nutritional importance of almond fruit is related to its kernel. Other

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parts of fruit such as shells and hulls were used as livestock feed and burned as fuel.^[4] Almond is a good source of protein, which is high in Arginine and possesses good digestibility^{[5],} almond is also highly nutritious, rich in monounsaturated fatty acids (MUFA), and packed with a wide variety of vitamins and minerals. The U.S. Food and Drug Administration (FDA) has defined almond as an excellent source of vitamin E and manganese. ^[6] Energy provided by almond is basically from its fat content, which is mainly composed of MUFA, particularly oleic acid. In addition to its nutritional value, almond also contains a number of phytochemicals that are responsible for a variety of bioactivities. among which antioxidant activity has been frequently studied.

The nutritive value of sweet almond *Prunus amygdalus*, were used in feeding infants in Lebanon, were studied for the aspects of protein quality and the possible presence of toxic factors.^[7]

Reports on the chemical composition and biological activities of almond were done ^[8] and nothing could be found on this cultivar grown in Egypt. This encouraged the authors to perform the present study aiming to throw light on the nutritional profile title plant.

MATERIAL AND MTHODS

Determination of macronutrients:

Proximate analysis of the kernel *Prunus amygdalus* B. includes the determination of moisture content, total ash, total protein, total fats, total carbohydrates and crude fibers. This analysis was done in accordance with the following references:

1-Nutritive value: was determined by multiplying the values obtained for protein, fat and carbohydrate by 4.00, 9.00 and 4.00 respectively and adding up the values.^[9]

2-Moisture content: was determined by drying about 2 grams of the kernels at 105°C until particularly constant weight was obtained. This required about 16 hours in an oven at 105°C. ^[10]

3-Total ash: 2 grams of the kernels, (0.5) g that accurately weighed, were heated in a crucible at 100°C until water was expelled.

The residue was heated slowly over a flame until swelling ceased. The crucible was then left in a muffle furnace at about 505°C to a constant weight.^[11]

4-Total carbohydrates:

A-Determination of Total Hydrolysable Carbohydrate: A known weight (0.2-0.5g) of the dried ground kernels was placed in a test tube, and then 10 ml of 1N sulfuric acid was added. The solution was then filtered into measuring flask (100 ml) and completed to 100 mls with distilled water.

The total Hydrolysable Carbohydrate was determined with the phenol-sulphuric acid method according to method of **Dubois et al**, **1956**.^[12]

B-Determination of total soluble sugars: Dried ground kernels (0.5) g were accurately weighed and extracted by boiling in 80% neutral aqueous ethanol for 6 hours. The extract was filtered, then the ethanol was removed by vacuum distillation, then the residue was completed to 50 ml with distilled water in a measuring flask. Total soluble sugars were determined in the extract using the phenol-sulphuric acid method according to method of **Dubois et al, 1956**^[12] as follows:

To 1 ml of aqueous sugars extract, 1 ml of 5% phenol solution was added and mixed. Then, 5 ml of sulphuric acid (98%) was added from a vast delivering pipette. The blank experiment was carried out by using distilled water instead of sugar solution. The tubes were shaken and allowed to stand for 10 minutes. The absorbance of yellow orange colour was measured at 490 nm. A stand curve was carried out by using pure glucose.

5-Total fats: The method of **A.A.O.C.** (1995) was conducted for lipid extraction from the kernels using chloroform: methanol (2:1 v/v) to extract the lipids. ^[10] The associated non-lipids were removed by washing lipid extract three times with methanol: water (1:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulphate, and then the solvent was removed by heating at 80°C under vacuum.

6-Total proteins: The total protein content of the samples was determined by Kjeldahl method according to **A.A.O.C.** (1995)^[13]; the

samples were heated with sulphuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. The solution is then distilled with sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample, is determined by back titration. The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid then titrated with a sodium carbonate solution using methyl orange as a PH indicator.

Study of vitamin E content

Vitamin E content of the kernel of *Prunus amygdalus* Batsch was determined by HPLC described by **Pyka and Sliwiok** (2001).^[14]

Apparatus for HPLC analysis of vitaminE

A Hewlett-Packard Series 1,100 liquid chromatography system (Waldbronn, Germany) equipped with a loop (20 μ l) diode array detector and a lichrosorb RP 15 column (4.0 mm i.d.x 250 mm; particle size 5 mm) (Merck, Darmstadt) was used. Elution was performed at a flow rate of 1 ml / min with mobile phase of water / acetic acid (98: 2 v/v, solvent A) and methanol / acetonitrile (50: 50, v/v, solvent B), starting with 5 % B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, 100% at 55 min, and kept at this stage for 5 min. A re-equilibration time of 15 min was then required. Quantitation was achieved at 292 nm by internal standard method. ^[15] Vitamin E content was determined according to the method described by Beyer and Jensen (1989).^[16]

Determination of the mineral content:

The mineral content was determined according to **A.A.O.C.** (1995). ^[13] Samples were digested by wet digestion with concentrated sulfuric acid in the presence of digestion catalysts (a mixture of copper sulfate and anhydrous sodium sulfate (1:10), and then the resulted solution was measured using Atomic absorption spectrometer.

Determination of the amino acids:

Amino acids were estimated by adopting the method of **Cosmos and Simon**-

Sarkadi(2002)^[17], the dried and defatted grinded kernels of Prunus amygdalus Batsch. 0.2 grams were hydrolyzed with 10 ml of 6N hydrochloric acid in a sealed tube, heated in an oven at 100°C for 24 hours. The resulting solution was completed to 25 mls distilled water and filtered, 5 mls of hydrolysate were evaporated until it was free from HCl vapors. Then the residue was dissolved in diluting sodium citrate buffer (PH 2.2) (composed of citric acid 14 g/L, sodium chloride 11.5 g/L, thiodiglycol 5 ml/L and sodium azide 0.1 g/L). The amino acids were eluted by the special programmed buffer system with a flow rate of 0.3 ml/min. The effluent was met by a stream of ninhydrin reagent at a flow rate of 0.2 ml/min, the mixture being passed through a high temperature reaction coil at 121°C where the color was produced. The absorbance of the colored compounds was measured continuously by a photometer at 570 and 440 nm. The results obtained were compared with those of a standard solution of amino acids. Determination of free amino acids was confirmed by comparing the retention times and peak areas of amino acid standards with those of the components present in the sample.

RESULTS

Determination of macronutrients:

The proximate analysis of the kernel of *Prunus amygdalus* Batsch are illustrated in **(table1)**

Study of vitamin E content

The content of vitamin E in almond nuts analyzed was (77.67 mg/100gm).

Determination of the mineral content:

The results of the mineral content of the kernels of *Prunus amygdalus* Batsch are compiled in (**table2**).

Study of amino acid content:

The amino acid profile of *Prunus amygdalus* Batsch Kernels as compiled in (table3) showed that the identified amino acids amounted to (40.2%) calculated on dry basis. Total non-essential amino acids percentage (70%) was much higher than the percentage of the essential amino acids (30%). Glutamic acid (5.02%) was the major non-essential amino acid identified, followed by aspartic acid (2.29 %) and Arginine (2.18 %), whereas Cysteine (0.37%) was the minor component. Concerning the identified essential amino acids, Leucine was the major one (1.38 %), while methionine (0.16 %) was the minor component. The ratio of lysine/arginine was very low (0.26).

DISCUSSION

Determination of macronutrients:

Although calorie-rich, tree nuts are a good source of several nutrients including proteins. certain vitamins and minerals, and several bioactive components such as sterols. The high calories in tree nuts are mainly due to their high lipid content. ^[18]Almond showing a total fat (52.083%) which indicating it is a good oil source. also the protein constitute (22.83 %), showing that almond are nutritiously and suggest that it can contribute to the daily protein need for adults as recommended by the Dietary Reference Intakes. almonds show a considerable amount of carbohydrate (16.9%) adequate to supplement other sources.

Almond shows a low moisture content (4.37%) which is important for an extended shelf life and sensory quality of nuts, as low moisture helps reduce microbial growth and various undesirable biochemical changes that often accompany it and the ash content which is relatively low (3.82 %).

These results, thus give an indication that almond are rich source of energy (627.647 Kcal.) and are capable of supplying the daily energy requirements of the body.

Study of vitamin E content

The content of vitamin E in almond nuts analyzed (77.67 mg/100gm) comes in higher content than those listed in USDA National Nutrient Database for Standard Reference, 26mg/100gm.

Vitamin E is well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant, protecting cell membranes from peroxidative damage. Free-radical-mediated pathology has been implicated in the development over time of degenerative diseases and conditions.^[19]

Determination of the mineral content:

The results of the mineral content of the kernels of *Prunus amygdalus* Batsch are compiled in (**table2**). The determination of minerals and trace elements in food stuffs is an important part of nutritional and toxicological analyses.

The macronutrients (quantity essential elements) such as sodium, potassium and calcium are present in Prunus amygdalus Batsch kernels in considerable amounts, where the concentration of sodium is (1072.5 is mg/kg), which a relatively low concentration, this is considered beneficial as although, sodium is a part of everyone's diet, but sodium intake is one factor involved in the development of hypertension.

On the other hand, foods rich in calcium and potassium are strongly recommended as protective measures against hypertension as recommended by the American Heart Association. ^[20]Fortunately, almonds contain relatively high concentrations of calcium, potassium and sodium.

Calcium, the most abundant mineral in the body, is an important component of a healthy diet and a mineral necessary for life. Calcium is required for vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling and hormonal secretion. Long-term calcium deficiency can lead to rickets and poor blood clotting and in case of a menopausal woman; it can lead to osteoporosis. [21] Therefore. Prunus amygdalus Batsch (containing 0.23 %) that equal mainly 2300 mg/kg would count in the recommended daily intake of calcium.

Potassium plays an important role in muscle contraction and nerve transmission. When the movement of potassium is blocked or when potassium is deficient in the diet, activity of both muscles and nerves can become compromised. Also, potassium is involved in the storage of carbohydrates for use by muscles as fuel. It is also important in maintaining the body's proper electrolyte and acid-base (PH) balance.^[22] Potassium may also counteract the increased urinary calcium loss caused by the high-salt diets. ^[23]*Prunus amygdalus* Batsch is considered as a good source of potassium (6656 mg/kg).

Almond contains high quantity of Magnesium (2864 mg/kg), this element is required for processing ATP and for bones and Recommended Dietary Allowance is 450 mg, so almond considered as good daily source of Magnesium.

With regard to minerals, the nutritional interest of almond is mainly due to trace elements such as Selenium, Iron, Copper, and zinc. Generally, the order of the concentration of the elements in Prunus amygdalus Batsch kernels sample is found to be Fe> Se > Zn> Cu. These elements are essential micronutrients for human health. In addition, they play an important role in human metabolism, and interest in these elements is increasing together with reports of relationships between trace elements status and oxidative diseases.^{[24][25]}

Higher Copper content of *Prunus amygdalus* Batsch (13.55 mg/kg) is higher than RDA although it is relatively lower content, compared to previous researches^{[26] [27]}, this element is essential as it can be found in many enzymes, including those involved in energy production, connective tissue formation, central nervous system function, antioxidant functions, melanin formation, gene expression and iron metabolism.

Also, various studies have reported a direct correlation between the dietary Zn/Cu ratio and the incidence of cardiovascular disease. However, gaining adequate zinc is important, because it plays critical roles in neurological functions, immune response, growth and development, and reproduction. ^[28]*Prunus amygdalus* Batschis considered as a good source of zinc (52.25 mg/kg).

Among many roles with which selenium (Se) was accredited, the antioxidant effect is the first one.^[29]Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals.^[30] The content of selenium in almond Umm alfahm cultivar nuts analyzed (68.71mg/kg) comes in higher content than in the previously reported data and higher than RDA.

Study of amino acid content:

The amino acid profile of *Prunus amygdalus* Batsch Kernels as compiled in (table3) confirms the earlier observation that almond contains a relatively low content of lysine and high levels of arginine.^[31] The high levels of arginine in almond (2.18%) has already been identified as a positive feature because arginine can be converted into nitric oxide a potent vasodilator which can inhibit platelet adhesion and aggregation.^[32] A low ratio of lysine/arginine in protein has also been identified as a positive feature in the of the development reduction of atherosclerosis in laboratory animals.^[33]

Finally, it is note-worthy to mention that these data could be useful criteria for the identification of *Prunus amygdalus* Batsch cultivated in Egypt. This is the first report on the nutritional profile of its kernels. The data reported in this work confirms that the kernels of *Prunus amygdalus* Batsch cultivated in Egypt are good for health and should readily be incorporated into the diet.

This is the first report on the nutritional value of kernels of Umm alfahm cultivar. The data reported in this work confirms that the kernels of *Prunus amygdalus* Batsch cultivated in Egypt are good for health and should readily be incorporated into diet.

REFERENCES

1. Egyptian Ministry of Agriculture statistics (2009): p 354

2.Namli S,Işikalan C, Akbaş F, Başaran D (2011): Improved in vitro rooting of almond (Amygdalus communis) cultivar "Nonpareil"., Plant Omics Journal, 4(1): 14-18.

3.**Takeoka G, Dao L, Teranishi R, Wong R, Flessa S, Harden L, Edwards, R** (2000):Identification of three triterpenoids in almond hulls., Journal of agricultural and food chemistry, 48: 3437-3439.

4.**Esfahlan AJ, Jamei R, Esfahlan RJ (2010):** The importance of almond (Prunus amygdalus L.) and its by-products, Food Chemistry, 120: 349– 360.

5.Ahrens S, Venkatachalam M, Mistry AM, Lapsley K, Sathe SK (2005):Almond (Prunus dulcis L.) protein quality., Plant foods for human nutrition (Dordrecht, Netherlands), 60: 123–128.

6.**Chen C, Milbury PE, Lapsley K, Blumberg JB (2005):** Flavonoids from Almond Skins Are Bioavailable and Act Synergistically with Vitamins C and E to Enhance Hamster and Human LDL Resistance to Oxidation, The journal of nutrition, 135: 1366–1373.

7.Cowan JW, Sabry ZI, Rinnu FJ, Campbell JA (1963): Evaluation of Protein in Middle Eastern Diets, The journal of nutrition, 81: 235–240.

8.Duke JA and Wain KK (1981): Medicinal plants of the world, Computer index with more than 85,000 entries. 3 vols.

9.Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M (2005): Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Curr. Sci., 89: 1252-1255.

10.A.A.O.C. (2000): Official methods of analysis of Association of Official Analytical Chemists, 17th edition, Washington, D.C.

11.A.A.O.C. (**1986**): Official methods of analysis of Association of Official Analytical Chemists, 14th edition, Washington, D.C.

12.Dubois M, Gilles K, Hamilton J, Rebers P, Smith F (1956): Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3): 350–356

13.A.A.O.C. (**1995**): Official methods of analysis of Association of Official Analytical Chemists, 16th edition, Washington, D.C.

14.Pyka A and Sliwiok J (2001): Chromatographic separation of tocopherols, Journal of chromatography, 935: 71–76.

15.Evangelisti F, Zunin P, Tiscomia E, Petacchi R, Drava G, Lanteri S (1987): Stability to oxidation of virgin olive oils as related to olive conditions: study of polar compounds by chemiometric methods, J. Am. Oil Chem. Soc., 74: 1017-1023.

16.Beyer RS, and Jensen LS (1989): Overestimation of the cholesterol content of eggs, Journal of Agriculture and Food Chemistry, 37: 917-20.

17.Cosmós E and Simon-Sarkadi L(2002): Characterization of Tokaj wines based on free amino acids and biogenic amines using ionexchange chromatograph, Chromatogrphia, 56: 185-188.

18. **Shahidi F, Zhong Y, Wijeratne SSK, Ho C** (**2009**): Nuts Composition, Phytochemicals and Health Effects, naturaceutical science and technology, series 9 Edited by Cesarettin Alasalvar, Fereidoon Shahidi, CRC Press, 127-141

19.Packer L (1991): Protective role of vitamin E in biological systems, Am J Clin Nutr., 53,(4):1050S-1055S.

20.He FJ, MacGregor GA (2009): A comprehensive review on salt and health and current experience of worldwide salt reduction programmes, J. Hum Hypertens, 23(6): 363-84.

21. Food and Nutrition Board, Institute of Medicine (2010): Dietary reference intakes for calcium and vitamin D, Washington, DC: National academy Press.

22.Sigworth FJ (2001): Potassium Channel Mechanics, Neuron, 32, 4: 555-556

23.Sellmeyer DE, Schloetter M, Sebastian A (**2002**): Potassium Citrate Prevents Increased Urine Calcium Excretion and Bone Resorption Induced by a High Sodium Chloride Diet, 87 (5):2008–2012

24.Pelus E, Arnaud J, Ducros V, Faure H, Favier A, Roussel AM (1994): Trace element (Cu, Zn, Fe, Mn, Se) intakes of a group of French men using the duplicate diet technique. Int J Food Sci Nutr , 45: 63 –70

25.Fennema, O. R(2000): Food chemistry, New York, Marcel Dekker

26.Aslanta R, Güleryüz M, Turan M(2001): Some chemical contents of selected almond (Prunus amygdalus Batsch) types, ciheam-iamz, 347-350.

27.Shahidi F, Zhong Y, Wijeratne SSK, Ho C(2009): Nuts Composition, Phytochemicals and Health Effects, naturaceutical science and technology, series 9 Edited by Cesarettin Alasalvar, FereidoonShahidi, CRC Press, 1-36

28.Laura D, Byham-Gray, Jerrilynn D, Burrowes, Glenn M, Chertow (2008): Nutrition in Kidney Disease, springer.

29.Cosmulescu SA, Baciu G, Achim M, BotuTrandafir I (2009): Mineral Composition of Fruits in Different Walnut (Juglans regia L.) Cultivars, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2):156-160.

30.Thomson C D (2004): Assessment of requirements for selenium and adequacy of selenium status: a review, European Journal of Clinical Nutrition 58:391–402.

31.Venkatachalam M, and **Sathe SK (2006):** Chemical Composition of Selected Edible Nut Seeds, Journal of agricultural and food chemistry, 54 (13): 4705–4714.

32.Kritchevsky D (1982): Trans-fatty acid effects in experimental atherosclerosis, Federation Proceedings, 41: 2813–2817.

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Item	Percentage
Moisture content	4.372
Total ash	3.82
Total protein	22.83
Total fat	52.083
Total carbohydrates [*]	16.895
Crude fibers	8.97
Nutritive value	627.647 Kcal.

Table 1: The	proximate anal [,]	vsis of the k	ernel of <i>Prunus</i>	amygdalus Batsch
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*calculated by difference

Table 2: Minerals content of the kernels of Prunus amygdalus Batsch

Mineral	Essentiality	Amount
Potassium ^a	Quantity elements	6656
Calcium ^B		0.23
Sodium ^a		1072.5
Magnesium ^a		2864
Phosphorus ^b		0.53
Iron ^a	Essential trace elements	119.9
Zinc ^a		52.25
Selenium ^a		68.71
Copper ^a		13.55

^a amounts are expressed as mg/kg fresh weight, ^b amounts are expressed as a percent (%).

Table 3: Percentage of amino acids content in the kernels of Prunus amygdalus Batsch

Amino acids	Conc. (%)
Leucine (e)	1.38
Phenyl alanine (e)	1.26
Valine (e)	0.92
Isoleucine (e)	0.74
Threonine (e)	0.6
Lysine (e)	0.56
Histidine (e)	0.54
Methionine (e)	0.16
Glutamic acid (n)	5.02
Aspartic acid (n)	2.29
Arginine (n)	2.18
Glycine (n)	1.28
Alanine (n)	0.93
Proline (n)	0.84
Serine (n)	0.82
Tyrosine (n)	0.66
Cysteine (n)	0.37
Total essential amino acids (e)	6.16
Percentage of total essential amino acids	30
Total non-essential amino acids (n)	14.39
Percentage of total non-essential amino acids	70
Total determined amino acids	20.55
total protein	22.83

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(e): essential amino acid, (n): non-essential amino acid.