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Grape Pomace Flour (Vitis spp.) from Shiraz in South of Iran by High Trace Mineral Elements as Food Supplements

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Abstract

The categorization of grape pomace is difficult. More than other food industries, fruit juice manufactured in Asia and wine-making practices in Europe and Australia and USA are incredibly diverse. Grapes are among the most economically important horticultural crops with a total worldwide production of 67 000 000 MT. Iran has an annual output of more than 2 million metric tons. Iran has a special position in Asia which has been creating a diverse ecosystem. In this project determination of ion concentrations and especially trace and mineral elements as nutritive value and considering grape fruit as a common popular fruit were highly regarded by the beneficial effects on human health and its economic importance which is widely grown and eaten not only in Iran but around the world. Accessions Khosnau, Red Rishbaba, Shahroudi, Shoush Aroos, Yaghouti, Askari were analyzed in June of 2017. The collection sites are located in the villages and cities: Zarqan; Jahrom and Mamasani near Shiraz in Fars province in the south of Iran. After extraction, the grape pomace samples were oven dried and being floured. The mineral and trace elements concentrations and nitrate and nitrite and physicochemical compositions were studied according to international protocols. In current study, the nutritive value of 5 different grape pomace native to south of Iran, in Fars province was determined and results revealed that it is so rich in crude protein, Lipids and some essential mineral elements especially Potassium, Calcium, Zinc, Copper Iron, Manganese. Our results suggested that it could be recommended as a dietary supplement for people who need essential mineral elements. In conclusion the present study revealed that the flour of grape pomace growing in south of Iran could be a new source of high protein and mineral elements and its full potential should be exploited. The utilizing of this flour is of potential economic benefit to the poor native population of the areas where it is cultivated. Therefore due to the high protein, trace and minerals of different high qualities studied grape and their flour of pomace be a new source of edible supplement after the future toxicological studies.

Keywords

Grape Pomace Flour; Mineral elements; Shiraz; Food Supplement; Iran

Introduction

Grapes are among the most economically important horticultural crops with a total worldwide production of 67 000 000 MT [1]. China is the leading producer followed by USA. Turkey ranks 6th in the world in total grape production and has the 4th largest vineyard area in the world after Spain, France and Italy [1].

Grapes are classified as table grapes, wine grapes, raisin grapes and juice grapes [2]. Global grape production currently amounts to more than 75 million

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metric tons per year. Today there are well over 18 million acres of cultivated vineyards worldwide. Italy produces the majority of the world's grapes, with an annual grape production of 8,307,514 metric tons. France and the United States aren't far behind, with annual productions of 6,740,004 and 6,206,228 metric tons, respectively [2, 3]. Spain and China each produce well over 5 million metric tons each year. Turkey produces 3,763,544 metric tons annually. Argentina, Iran, and Chile have an annual output of more than 2 million metric tons, and South Africa produces a solid 1,587,913 metric tons each year [2, 3]. Iran and Turkey are two countries with high levels of grape production despite the fact that very little of Turkey's population consumes wine, and wine consumption is illegal in Iran. But both countries have an ideal climate for grape cultivation and produce massive amounts of table grapes and raisins [3].

Grapes are consumed both as fresh and as processed products such as wine, jam, juice, jelly, grape seed extract, dried grapes, vinegar and grape seed oil. The cultivation of grapes is widely spread around the world with an estimated surface area of 7.5 million hectares in 2014. Of this, 39% was produced in Europe, 32% in Asia and 20% in America [3]. Much of the world's grape cultivation is intended for the production of wine, and nearly all of the countries on this list are among the world's top wine producers. Over 60% of the world's wine is consumed within Europe, with Italy, Spain, and France dominating the world's wine export market. Over half of the world's wine is exported from these three countries [3].

The residue of grape agro-industrial is mostly solid by-products such as pomace, stalks and the liquid filtrate. According to the conditions of the grapes when they are harvested, the residues may represent from 13.5 to 14.5% of the total volume of grapes, and may reach 20% [4, 5].

The categorization of grape pomace is difficult. More so than other food industries, fruit juice manufactures in Asia and wine-making practices in Europe and Australia and USA, are incredibly diverse, and, while rooted in tradition, they have been embracing changes required both by globalized consumer demand and by legislative pressure to reduce their environmental footprint. As a result, the physical and chemical composition of fruit juice and raisins and other by-products depends on many factors: purpose of the crop (spirits, juice, etc.), grape variety, grape maturity, and the wide range of techniques and machinery used throughout the process [6]. Indeed, the nature and nutritional value of those products should be ideally determined on a case-by-case basis. Grape pomace is a fresh (up to 65-68% water) and perishable product, and it must be dried if it cannot be fed immediately or ensiled [7]. Grape pomace is usually dried in large rotary drum driers. Pomace is introduced at the top where a large hot air fan is situated. The hot air is forced through the pomace, expelling gases and moisture vapor out of the drum drier. The wet pomace dries as it rotates through the drum, the supply of hot air being maintained by the drum burner. The dried pomace is then hammer-milled into a fine mash, which may be mixed with small amounts of molasses to improve its energy content and its palatability. Addition of lime can be used to bind the pectines and raise the pH [8]. In a process described in Australia, drying occurs continuously for approximately 20 minutes, in a gas-fired rotary drum heated to approximately 120°C. The resulting dry meal is ground and then steam-pelleted at 85°C [9].

Many by-products from food industries including grape pomace are generally used as animal feed or fertilizers. Grape pomace is considered as a valuable by-product for oil extraction, phenolics, tocopherols and antibacterial agents. Actually, the waste products could provide supplements for the food and drug industry. Our objective was to determine details of the nutritive values of grape pomaces of top often commercial Persian grape varieties to consider possible applications in the food and feed industry and even utilizing as bio-adsorbent for removing heavy metals from contaminated soils. I current study, some chemical properties and minerals contents, namely potassium (K),lithium(Li) , calcium (Ca), magnesium (Mg), sodium (Na), phosphorus (P), iron (Fe), zinc, copper, selenium, cobalt, chrome, lead and cadmium, Arsenic, Nickel of the grape pomaces were investigated. The results indicated that grape pomaces were generally acidic in nature with pH values of 3.65-4.78. Pomace is usually poor in sugar content and the variety of grape significantly affects the sugar content of the pomaces. Crude fiber was the major constituent of the grape pomace and ranged between 50.17 and 63.28%. Mineral analyses also showed that pomace samples had high Zn, Cu, K, Ca, Mg, Na, P and Fe contents. Consequently, the pomace samples of the grape varieties could be considered functional foods with high biological values.

There is a clear correlation between plant communities, chemical composition and soil conditions. Iran has a special position in Asia which has been creating a diverse ecosystem [10].

As Iran is a region with full saline areas, ion concentration changes cause various soil qualities [11]. Therefore, in this project determination of ion concentrations and especially trace and mineral elements as nutritive value and considering grape fruit as a common popular fruit were highly regarded by the beneficial effects on human health and its economic importance which is widely grown and eaten not only in Iran but around the world.

Material and Methods

Sample Collection

Accessions Khosnau, Red Rishbaba, Shahroudi, Shoush Aroos, Yaghouti, Askari were used in current study. The collection sites are located in the villages and cities: Zarqan; Jahrom and Mamasani near Shiraz in Fars province which is shown in figure 1. All samples collected from different farmlands during commercial ripening stage varying between 10 to 30 June of the year 2017. Fars province is located in the south of Iran. It neighbors Bushehr Province to the west, Hormozgān Province to the south, Kerman and Yazd provinces to the east, Isfahan province to the north and Kohgiluyeh and Boyer-Ahmad Province to the northwest.

After harvest, the grapes were transported in cool boxes to the city of Tehran, capital of Iran to the Laboratory of Nutrition and Food Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, where they were stored under refrigeration for 48 hours at 4°C until analysis. Initially, the grapes were weighed and graded according to Institute of Standards and Industrial Research of Iran. 2009.

Grape juice-Specifications and test methods, 1815 standard, 3th Revision [12]. The grapes used were from Group I (consisting of variety with seed), Subgroup Colorful, Class 2 (mass of clusters between 200g and 500g), and Extra Class (typical coloring, green stems, and no bunch deformation). Then grapes were then cleaned and pressed using an industrial depulper separating the pomace (skin and seeds) and pulp for the extraction of grape juice. The juice of the all studied grapes was studied individually and stored at (-20 °C) until analyzed for the following organic acids of succinic, citric, malic, fumaric and tartaric [13].

Figure 1: Sitemap of sample location

After extraction, the grape pomace samples were stored in polyethylene bags at −18 °C until processing. The pomace samples individually were dried in an air circulation oven (China DGG-9070B Stand-Drying and *Air Circulation Oven*) at 70 °C for approximately 12 hours.

The dried residues were grinded using a *Tefal* GT203840 Coffee *Grinder* with Twin Cutting Stainless Steel Blades, and the flours were achieved and sieved using a set of seven sieves Analchem DIN-4188 (10, 15, 20, 22, 25, 28,32 and 36 mesh corresponding to openings: 0.010,0.015,0.020,0.022,0.025,0.028, 0.032 and 0.036 mm, respectively). The flours were packed in covered polyethylene bottles until analysis.

Then studied flours conducted to chemical analysis for determination of some chemical composition such as studying: vitamin C, anthocyanins, mineral and trace elements and toxic potential. Afterwards, from the flours obtained, extractions utilized in different solvents were performed and the extracts were subjected to toxicological analysis.

Zinc, Manganese, Copper and Potassium Determination

For Zinc, Manganese, Copper and Selenium concentration of grape pomace samples were dried in oven for 68-72 hours at a temperature of 85°C. The samples were then ground and sieved through 0.5 mm sieve. The powdered samples then subjected to the acid digestion using nitric acid (65% Merck, Germany), Sulfuric acid (96.5% Merck, Germany) and perchloric acid (70% Sigma-Aldrich). Two gram of air-dried of each homogeneously grape pomace samples accurately weighed and 20.0 mL of the digestion mixture (3 parts by weight of concentrated nitric acid: 2 parts of concentrated Sulfuric acid & 3 parts by weight concentrated perchloric acid) and heated slowly by an oven and then rise the temperature [14-29]. The remaining dry inorganic residues were dissolved in 25.0 mL of nitric acid and the solution used for the determination of mineral elements. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents [21-29]. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame, using at least five standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis [26]. Also, periodic testing of standard solutions was performed in order to verify of reliability of the measuring apparatus. The accuracy was checked using quality control test for fungi and their substrate samples to show the degree of agreement between the standard values and measured values; the difference was less than 5%. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene, flame temperature: 2800°C, acetylene pressure: 0.9–1.0 bar, air pressure: 4.5–5 bar, reading time: 1–10 sec (max 60 sec), flow time: 3-4 sec (max 10 sec).

Iron Determination

The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 mg /L) were placed in stepwise volumes in 100 ml volumetric flasks. 2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times [21-28].

Calcium, Sodium and Magnesium Determination

The contents of Ca, Mg and Na in walnut kernels were measured by atomic absorption spectrophotometer (AAS) (Model AA-6200 Shimadzu, Japan) according to the method of Hernandez [31-34]. A 5 g sample was placed in a previously weighed porcelain crucible and ignited The resulting white ash was weighed, dissolved in 12 ml of concentrated nitric acid, percholoric (3:2) and diluted with nitric acid 10% in a 25 ml calibrated flask. The solution then was used to determine Ca, Na, and Mg. Standard stock solution of sodium, magnesium and calcium was prepared from AAS grade chemicals (Merck, Germany) by appropriate dilution [19-30].

Acidity, pH

Acidity was determined by titration with 0.1 N NaOH and the results were expressed in grams of citric acid/100g. The pH was determined by direct reading on the potentiometer, (MS Tecnopon, model mPA210) calibrated in buffer solutions of pH 4.0 and 7.0. One gram of the oven-dried samples in powder was placed in acid washed crucible by known weight. They were ignited in a muffle furnace for 2-3 hours at 550 ºC. After cooling crucibles they were weighed and the ash contents were expressed in terms of the oven-dried weight of the sample [31-33].

Moisture Content

All samples were oven dried at 80 ^{o}C for 36-48 hours until a constant weight were obtained. The moisture contents were expressed as difference in the mass of the sample before and after drying; the result was expressed in percentage of moisture [31-34].

Crude Fiber

Five grams of the ground pomace samples were digested in 100 ml of 1.25% H_2SO_4. The solutions were boiled for 45 minutes and then were filtered and washed with hot distilled water. The filtrates were digested in 100 ml of 1.25% Sodium Hydroxide solutions. These solutions were heated for 60 minutes, filtered and washed with hot deionized water and over dried. The final oven-dried residues were ignited in a furnace at 550^{o}C. The weights of the left after ignition were measured as the fiber contents and were expressed in term of the weights of the samples before ignition [34].

Crude Protein

The protein nitrogen in one gram of the dried samples were converted to ammonium sulphate by digestion with concentrated H_2SO_4 (Merck 96.5%) and in the presence of $CuSO_4$ and K_2SO_4 [24, 25]. The solutions were heated and the ammonia evolved were steam distilled into Boric acid 2%. The nitrogen from ammonia were deduced from the titrations of the trapped ammonia with 0.1M HCl with Tashirus indicator (methyl red: methylene blue 2:1) until a purplish pink color were obtained. Crude proteins were calculated by multiplying the value of the deduced nitrogen by the factor 6.25 mg [34].

Quantitative Determination of Nitrate and Nitrite

a)Quantitative determination of nitrate

Fifty to seventy mg of sub-sample was selected and deionized water was added to the samples (nine times than exact the sample weight) and the water and sub-sample were homogenized for 15 minutes [36-40]. A 30 gram sample of homogenate was placed in a centrifuge tube, and 0.5 ml of H_2O_2 was added and the tube was capped and shaked well by the hand after adding H_2O_2. All samples were centrifuged at 3500 rpm for 3 min. The supernatant was then separated and filtered with filter paper watt man No 1 and nitrate concentration in the filtrate was determined calorimetrically by a flow injection analysis system [36]. Nitrate content was expressed as mg nitrate per kg on a fresh weight basis (mg NO_3/kg FW) unless otherwise stated. Nitrate concentration in celery was calculated from nitrate content in leaves and petioles separately on the weight of each part [36].

b) Quantitative Determination of nitrite:

According to the AOAC official Methods 973/31 determination of nitrite was done. A portion of solution containing nitrite was transferred into a 25 mL volumetric flask. Then 2.5 mL sulfonamide was added, followed by addition of 2.5 mL NAD (N− (1−naphthyl) ethylenediamin.2HCl). The volume was complete with water and left 15 minutes in order to give time for color development. The absorbance was measured at 545 nm against a blank solution. The nitrite concentration was determined using the calibration curve solutions of 0.1, 0.2, 0.4, 0.6, and 0.8 ppm $NaNO_2$. The absorbance values were measured at 545 nm. The calibration curve was constructed by plotting the absorbance vs. the concentration [36-39].

A Shimadzu (Model No: UV-2550) the double mono-chromator UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements.

Statistical Analysis

Values were expressed as the mean (mg/kg) ± standard deviation (SE) for Nitrate content differences on the basis of the type of fruit were determined by student t-test. The brand and manufactures differences were calculated by one way ANOVA and for analysis of the role of multiple factors univariate analysis was used by SPSS 20 .Probability values of <0.05 were considered significant.

Results

Results declared that approximately grape pomace is about 8% seeds, 9% stems, 25% skins, 58% pulp.

Table 1 :Mineral contents of pulps, seeds and peels of 5 different grape pomace (Red Rishbaba, Shahroudi, Shoush Aroos, Yaghouti and Askari, based on dry weight

Mineral elements	Mean± SD (mg/100g)
Ca	84.56±1.42
Mg	32.15±1.12
K	134.86±1.28
Fe	46.02±0.52
Zn	42.73±0.71
P	11.20±0.10
S	0.181±0.120
Li	0.011±0.001
Co	0.091±0.001
Cr	0.044±0.018
Cu	49.87 ±1.67
Mn	3.101±0.020
Na	0.136±0.018

Comparing with Dietary Reference Intakes (DRI) (National Academy of Sciences, 2011), the Iron content in grape pomace found in current study (46.02 mg/100g) supplies the adult daily requirements for iron (8mg/day for men and 8 to 18mg/day for women) [40-41], investigating minerals in grape juice, found values of sodium (0.067 mg/100 g), potassium (129.5 mg/100g), phosphorus (10.5 mg/100g), and magnesium (8.78 mg/100g), but lower values of iron (0.14 mg/100g). Sousa et al., [41] investigated the mineral content in grape pomace of (Benitaka) variety in Brazil and found that level of Calcium, Magnesium, Potassium and Iron were (0.44, 0.13, 1.40, 0.183 and 18.08 mg/100gm) respectively, these results were lower than that in our study except the iron content[41].

Due to the results of the mineral analysis shown in table 1, iron, copper, potassium, zinc, manganese, and calcium were present in much higher concentrations in comparison to other studies in Brazil and Libya [42]. There were no significant values for lithium. Potassium levels higher than those of sodium can lead to a mineral balance that favors hypertension control. A diet rich in potassium lowers blood pressure and consequently the risk of morbidity and mortality due to cardiovascular diseases; in addition, potassium intake can decrease urinary calcium excretion and consequently reduce the risk of developing osteoporosis [40]. The physicochemical of 5 different grape pomace (*Vitis Spp*.) flour samples were compared and results are shown in table 2according to % dry basis.

Table 2: Physicochemical analysis of grape pomace (*Vitis Spp*.) flour, Parameters (% dry basis and results expressed on (Mean ±SD)

Grape (*Vitis* spp.)	No. of Samples	Moisture (g/100g)	Ash (g/100g)	Total Lipids (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	Total dietary fiber (g/100g)
Red Rishbaba	34	3.40 ±0.28	4.73±0.14	8.23±0.14	8.56±0.06	28.01±0.23	46.44±0.18
Shahroudi	30	3.56± 0.11	4.78±0.07	8.15±0.21	8.66±0.09	27.65±0.19	47.20±0.14
Shoush Aroos	32	3.58±0.17	4.56±0.04	8.60±0.17	8.42±0.12	28.09±0.16	51.44±0.21
Yaghouti	30	3.87±0.13	4.62±0.16	8.15±0.16	8.34±0.15	28.83±0.07	46.19±0.17
Askari	38	3.42±0.11	4.71±0.14	8.33±0.09	8.19±0.13	27.67±0.15	47.68±0.17
Mean	164	3.57±0.16	4.68±0.11	8.29±	8.43±0.15	28.05±0.16	47.79±0.17

The mean content of nitrate and nitrites and their ranges in the grape pomace after preparing fruit juice and dried the pomace from different farmlands in Jahrom, Mammasani and zarqan in Fars province in June samples were determined as Dry weight ± standard error of the mean and shown in Table 3.

The result from current study showed in table 3 declared that all the grape pomace analyzed contained low amount of nitrate, nitrite. The mean nitrate and nitrite levels in the pomace after process of prepare juices ranged from 6.56-12.38 (mg/kg) for Shoush Aroos samples , 8.14-13.87 (mg/kg) for Yaghouti , 8.89-14.22(mg/kg) for Askari samples , 5.67-18.92 (mg/kg) for Shahroudi and 6.88-15.44(mg/kg) for Red Rishbaba **.** The mean was 8.33±0.12 (mg/kg± SE) which was the least for Shoush Aroos up to 12.64±0.55 (mg/kg± SE) for Shahroudi grape pomace samples respectively.

The different between mean values of nitrate in different grape pomace samples is statistically significant (p<0.05). The mean concentration of nitrate in Shahroudi samples are significantly higher than other pomace samples while mean nitrate content in Red Rishbaba 5.38±0.42 (mg/kg± SE) is significantly higher and Shahroudi and Shoush Aroos samples were the same and has no meaningful differences. Nitrate and nitrate contents in all pomace samples were much lower than maximum permissible level.

Conclusion

In this study, the nutritive value of 5 different grape pomace native to south of Iran, in Fars province was determined and results revealed that it is so rich in crude protein, Lipids and some trace and essential mineral elements especially Potassium, Calcium, Zinc, Copper Iron, Manganese and Phosphor. Traditionally the grape pomace used widely for the feeding of animals, but scientifically few study was done on nutritive value of flour of pomace and also practical utilizing of it has been yet screened out. The results of this research by revealing high mineral and trace elements content in 5 different *grapes* (*Khosnau, Red Rishbaba, Shahroudi, Shoush Aroos, Yaghouti, Askari*) culturing in Fars province needs more surveys on human health. Therefore the scientific studies should be conducted to investigate the unexploited potential of flour of grape pomace. Our results suggested that could be recommended as a dietary supplement for people who need essential mineral elements. In conclusion the present study revealed that the flour of grape pomace growing in south of Iran could be a new source of high protein and mineral elements and its full potential should be exploited. The use of this flour is of potential economic benefit to the poor native population of the areas where it is cultivated. Therefore due to the high protein, trace and minerals of different high qualities studied grape and their flour of pomace be a new source of edible supplement after the future toxicological studies.

Table 3: Mean nitrate (NO_3) content (mg/kg) in the grape pomace after preparing fruit juice and dried the pomace from different farmlands in Jahrom , Mammasani and Zarqan in Fars province in June 2017

Grape pomace samples	Mean(NO_3) mg/kg ± S.E*	Range of Nitrate content (mg/kg)	Mean(NO_2) mg/kg ± S.E*	Range of Nitrite Content (mg/kg)
Red Rishbaba 1	12.14 ± 1.15	9.06- 13.37	5.47 ± 0.34	2.18-6.08
Red Rishbaba 2	10.44± 1.12	10.01 -11.25	4.78± 0. 44	3.87-6.22
Red Rishbaba 3	12.65 ± 1.00	7..44- 13.09	6.42±1.08	3.56-6.78
Red Rishbaba 4	13.14 ±1.02	6.88-15.44	5.18 ± 0.06	4.00-6.11
Red Rishbaba 5	13.78±0.89	9.23-15.10	5.03 ±0. 16	3.98-6.89
Mean	^{b}12.43± 1.04	6.88-15.44	^{a}5.38±0.42	2.18-6.89
Shahroudi 1	13.28±0.39	10.18-15.66	3.22± 1.01	2.11-4.98
Shahroudi 2	8.78 ±0.91	5.67-11.01	2.39± 0.42	2.43- 3.29
Shahroudi 3	11.23±0.38	10.21-13.06	4.30± 0.76	3.03-5.18
Shahroudi 4	12.67±0.44	10.87-14.32	5.11 ± 0.42	3.28-6.78
Shahroudi 5	17.23±0.62	15.09-18.92	3.22± 0.27	2.89-4.17
Mean	^{a}12.64±0.55	5.67-18.92	^{b}3.65±0.58	2.11-6.78
Shoush Aroos1	8.12±0.17	6.78-9.23	2.23± 0.04	2.01-3.18
Shoush Aroos2	7.36±0.12	6.56-8.17	3.76 ±0.06	2.67-4.15
Shoush Aroos3	7.84±0.09	6.87-8.67	3.44± 0.06	2.78-4.87
Shoush Aroos4	10.07±0.11	7.42-12.38	3.47 ± 0.09	3.01-5.04
Shoush Aroos5	8.24±0.11	7.02-11.02	3.65± 0.06	2.76-4.11
Mean	^{e}8.33±0.12	6.56-12.38	^{b}3.31± 0.06	2.01-5.04
Yaghouti 1	12.01±0.02	9.86-13.87	2.08 ± 0.15	2.00-2.78
Yaghouti 2	10.28±0.08	9.76-13.67	2.04 ± 0.12	1.98-3.01
Yaghouti 3	10.83 ± 0.05	9.89-12.98	3.12± 0.08	3.01-3.87
Yaghouti 4	9.42±0.12	8.14-10.67	3.43 ± 0.06	2.87-3.76
Yaghouti 5	10.02 ±0.08	9.04-11.87	3.56± 0.09	1.98-3.87
Mean	^{d}10.91± 0.07	8.14-13.87	^{d}2.84±0.10	1.98-3.78
Askari 1	13.09 ± 0.15	10.44-14.20	3.04± 0.05	2.98-3.19
Askari 2	9.96± 0.23	8.89-10.11	3.28 ± 0.27	2.45-3.78
Askari 3	12.22±0.23	10.26-13.44	3.02± 0.13	2.68-3.19
Askari e 4	13.03±0. 11	10.28-14.22	2.78±0.08	2.18-2.98
Mean	^{c}12.07±0.18	8.89-14.22	^{c}3.03 ±0.13	2.18-3.78

Conflict of Interests

it is declared that the authors neither have any financial gain nor conflict of interests regarding this paper.

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