Fatty Acid Composition, Mineral contents and antioxidant activity of grape seed powder by (GC-MS), ICP/OES and DPPH method.

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Abstract

Among the largest fruit harvests in the world are grapes (vitis vinifera). This study's major objective is to describe grape seed byproducts and waste as novel sources of bioactive components from a circular economy and biorefinery perspective. The fatty acids included in grape seeds have been found to have strong free radical scavenging properties.in particular,

this research investigated :1.chemical composition of grape seed powder using the method of gas chromatograph with mass -spectroscopic detection (GC-MS),the obtained data allowed to establish that the studied (GSP) contains major constituents that are these fatty acid palmitic acid (14.83%),9-Octadecanoic acid(Z))-2,hydroxy ethyl ester(9.75%) ,linoleic acid (7.43%) ,oleic acid (21.31%), Linoleic acid and olieic acid are the two main fatty acids, though of fatty acids, grape seed powder include 4-Acetoxy-3-(2,6,11-trimethyldodeca-2,6,10-trienyl)benzaldehyde (6.02%),14-Beta-H-Pregna (7.64%) and Tricosane (14.99%) .the important of these component in medicine includes anti-viral and antioxidant properties. 2. Radical scavenging activity of (GSP) were evaluated by DPPH assay. Grape seed is known as an effective antioxidant that protects the body from premature aging and disease. EC_{50} of grape seed powder was 0.536mg/ml, radical scavenging activity increase from 2.6 to 49.13 by increasing concentration from 4.8µ/L to 1250 µ/L, indicate strong relation between concentration and radical scavenging activity.3. mineral contents of grape seed powder were detected using inductivity coupled plasma optical emission spectrometer (ICP/OES), according to the results (Ca, Al, Cu, and Fe) were established as the primary minerals with high concentrations, respectively. whereas very low levels of (Ni, Cd, Co, and Cr) were discovered.

Key word: Grape seed powder (GSP)); GS/MS; ICP/OES; Antioxidant activity; DPPH; S FA; PUF.

Introduction:

The wine industry uses around 80% of the grape harvest, which is a significant fruit production. [1]. 15% or so of the solid waste generated by the wine industry is made up of grape seeds. For both environmental and financial considerations, there has been an increase in interest in and research focusing on the composition of agrifood industry waste in recent years. Italy, France, and Spain are the market leaders in the viticulture industry, which is significant to the European economy [2]. Wine is the primary product of the oenological industry, but during the processing of grapes, waste and byproducts such as grape skin, pulp, seeds, and pomace are also created in large quantities. Alternative uses for these byproducts are being investigated, taking into account things like enhancing the environment, cutting manufacturing costs, and providing fresh avenues for industrial diversification [3,4]. Recent trends suggest that non-extracted products like pomace and grape seed flours could be of interest for utilizing the large variety of bioactive compounds. (Lucarini et al.) [5]. The grape skins and seeds collected after screening the pomace are the waste products of wine manufacturing that are particularly helpful for recovering bioactive compounds [6–13]. Cell wall polysaccharides, polyunsaturated fatty acids (PUFAs), pigments, proteins, phenolic compounds, and vitamins are among the

bioactive substances retrieved from wine trash that are particularly intriguing [14–16]. In grape seed, the PUFA/SFA ratio was 3, 17.

Additionally, the two main fatty acids were oleic acid (29%) and linoleic acid (52,26%). The following fatty acids can be found in grape seed oil according to its chemical makeup: oleic acid 15,4-15,6%, linoleic acid 71,7-73,1%, linolenic acid 0,3-0,6%, palmitic acid 7,2-8,5%, stearic acid 3,8-3,9% [17,18]. For example, grape seed extract (aqueous or alcoholic) has a high antioxidant potential; its positive effects include modulating the expression of antioxidant enzymes, protection against oxidative damage in cells, antiatherosclerotic and anti-inflammatory effects, and protection against some cancer types, in both people and animal models [19,20,21].

In recent years, the market for nutritional supplements has seen an increase in the popularity of grape seed extract, particularly in Australia, Korea, Japan, and the United States [22]. This is due to the abundance of phenolic chemicals in grape seeds [23] and may have positive benefits on human health, such as preventing peptic ulcers [23,24]. It has been noted that grape seeds can scavenge superoxide radicals [25]. The human diet contains some components that are crucial for maintaining healthy physiological processes [26]. However, certain substances, including as Ag, As, Be, Bi, Cd, Pb, and V, do not occur naturally in the body and have harmful effects when a high proportion of the corresponding beverages is consumed in a diet [27]. Health issues might result from an increase in the use of processed foods and a diet that is deficient in vitamins and minerals. Many people use dietary supplements as a means to get the important vitamins and minerals they need in their diet [28]. This preliminary study sought to investigate novel sources of functional components within this framework using a biorefinery technique and a circular economy perspective. A nutraceutical investigation was done on grape seeds. The investigation of the mineral contents and radical scavenging activities.

Material and Methods

Material

grape (*Vitis vinifera L.*) was obtained from sulaimani market in the May month, the grapes come from balad town that located in the north of the Baghdad city. The seeds after juice extracting with no any other parts of fruits products such as peels, at first grape seeds were separated from pomace, and subjected to the thorough purification (separation). The seeds were dried at 40˚C for 24h in an oven and ground to a powder. Then GSP (grape seed powder) was obtained by superfine grinded. Pomace, creat-ed after grape seed pressing was superfine grinded up to flour. (**Fig. 1**).

Fig1: Grape seed powder

Methods:

1- **Fatty Acids Analysis by Gas Chromatography- Mass Spectrometer (GC/ MS):**

The gas chromatograph analysis of fatty acids was performed on a Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25im film thickness) capillary column (intercut DB5MS . japan). two microliteres of sample were injected into the capillary column. Helium was used as the carrier gas. Injector and detector temperatures were set at 210°C.

Injection was performed in split mode (1:30). The column temperature was programmed initially at 50°C for 1 minute and then to increase at a rate of 3°C per minute to a final temperature of 210°C. Fatty acids were separated at constant pressure (100 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008

2 - Free radical-scavenger activity assessment (DPPH° assay):

The DPPH assay was performed according to the method developed by Kim et al. (2002) Extreme potential for scavenging was calculated according to the recorded technique [29]. At different concentrations, 1.5 ml of 0.25 m M DPPH mixture and 1.5 ml of extract were diluted in ethanol. For a constant state, this combination was vigorously shaken at ambient temperature. After thirty minutes DPPH decolorization was measured by taking a spectrophotometer to measure the absorbance at 517 nm. Then, the following equation was used to calculate how scavenging DPPH radicals works. Ascorbic acid was used as a positive control.

Scavenging activity equation $= [\frac{A0-A1}{A0} \times 100]$

A0 denoted the control absorbance (unqualified, with no extraction)

A1 Denoted the absorbance when the excerpt or regular example is present.

3- Determination of mineral contents using (ICP-OES).

About 0.5 g of finely ground grape seed samples was put into burning cup with 15 mL of pure HNO_3. The sample was incinerated in oven at 600 C^0. Distilled deionized water and ultra-high-purity commercial acids were used to prepare all reagents, standards and grape seed samples. After digestion the samples were filtrated through Whatman No. 42. The filtrates were collected in 50 mL Erlenmeyer flasks and analyzed by Inductivity Coupled Plasma Optical Emission Spectrometer (ICP-OES) (PerkinElmer Optima 2100 DV, USA). The mineral contents of the samples were quantified against standard solutions of known concentrations which were analyzed concurrently.

Results and discussion

1-Identification and quantification of chemical composition of (GSP) using (GC/MS).

The Gas-chromatography coupled with mass spectrometry analyzer was used to identify and quantify the grape seed powder sample; the chromatograms of the chemical components of grape seed are represented in Fig. 2.

On comparison of the mass spectra of the constituents with the NIST08 library the chemical compositions were characterized and identified in table 1.in the current study ,eight chemical constituents were detected in the grape seed powder includes ,saturated, monounsaturated and polyunsaturated fatty acids which accounted palmitic acid (14.83%) ,9-Octadecanoic acid (Z) -,2-hydroxyethylester(9.75%),4-Acetoxy-3-(2,6,11-trimethyldodeca-2,6,10-trienyl)benzaldehyde (6.02%),linoleic acid (7.43%),Oleic acid

(21.31%),Oleic acid (16.00%),cis-13-Octadecanoic acid(7.64%)and Tricosane (14.99%). As can be shown in table (2), the major components in GSP are fatty acids, among the identified unsaturated fatty acids, oleic acid is predominated, followed by linoleic acid, and one saturated fatty acid was detected is palmitic acid. As reported previously (Baydar and Akkurt,2001), grape seed were rich in linoleic acid and oleic acid. Unsaturated fatty acids, which are important to human health and cannot be produced by the body, have been found to reduce blood cholesterol levels, increase cell permeability, protect cardiac tissue, and prevent atherosclerosis [30]. Unsaturated fatty acid intake has several consequences on the body, including the ability to directly influence prostaglandin synthesis. Among them, linoleic acid deficiency results in metabolic problems because cholesterol binds to some saturated fatty acids. Linoleic acid is one of essential fatty acid for body, and it is suggested to consume for daily diet. Though the fatty acids, grape seed contain volatile compound (4-Acetoxy-3-(2,6,11-trimethyldodeca-2,6,10-trienyl)
benzaldehyde(6.02%),Tricosane(14.99%) and 14-b-H-Pregna(7.64%) .and the important of (paraffine hydrocarbon)are found in food preservatives and majorly in the petrochemical industry for production of candles, biopesticides, paraffin wax and other by-products of petroleum.

One constituent of grape seed powder is 14-b-H-Pregna, a steroid considered to be a sex pheromone specific to males, and also a defensive chemical with diabetic retinopathy prevention and treatment effects.

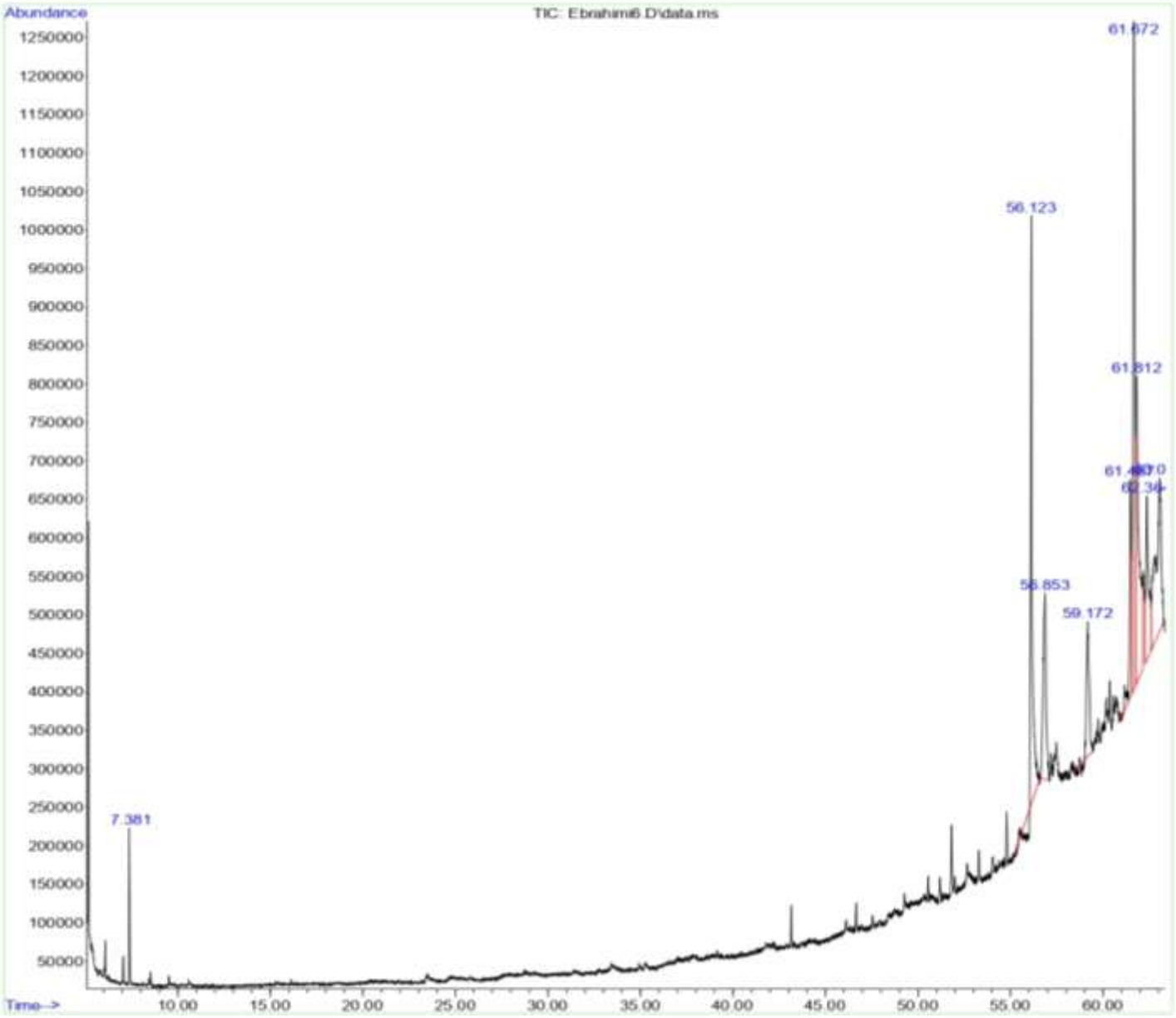

Fig 2: GC-MS chromatogram obtained for the grape seed powder

Table1: chemical components identified of Grape seed powder sample by GC/MS analysis.

Serial N.	**Compounds**	**Area %**	**Retention time**	**Molecular structure**	**Molecular weight**
1	Toluene	**2.04**	**7.379**	**C_7H8**	**102.15**
2	n-Hexadecanoic acid	**14.83**	**56.125**	**$C_{16}H_{32}O_2$**	**259.44**
3	9-Octadecenoic acid(Z)-,2-hydroxyethyl ester	**9.75**	**56.851**	**$C_{20}H_{38}O_3$**	**326.5139**
4	4-Acetoxy-3-(2,6,11-trimethyldodeca-2,6,10-trienyl)benzaldehyde	**6.02**	**59.171**	**$C_{24}H_{32}O_3$**	**368.5**
5	9,12-Octadecadienoic acid(Z,Z)	**7.43**	**61.486**	**$C_{18}H_{32}O_2$**	**280.4**
6	9-Octadecenoic acid(Z)	**21.31**	**61.669**	**$C_{18}H_{34}O_2$**	**282.4614**
7	9-Octadecenoic acid(E)	**16.00**	**61.812**	**$C_{18}H_{34}O_2$**	**282.4614**
8	14-BETA-H-PREGNA	**7.64**	**62.366**	**$C_{21}H_{36}$**	**288.5**
9	Tricosane	**14.99**	**63.069**	**$C_{23}H_{48}$**	**324.6**

Table 2: fatty acids composition in Grape seed powder by (GC/MS) analysis

Sample	C16:0 Palmitic	C18:1 Oleic 2-hydroxyethylester	C18:2 Linoleic	C18:1 Oleic	C18:1 Oleic
Rt	56.125	56.851	61.486	61.669	61.812
Ratio %	14.83	9.75	7.43	21.31	16

2-**Radical Scavenging Activity of Grape seed powder using DPPH assay.**

The most notable bioactive property of unsaturated fatty acid compounds is their antioxidant capacity. Using the DPPH technique, antioxidant activity of Grape seed powder was assessed. An electron or hydrogen radical can be accepted by the antioxidant impact, also known as DPPH, to create a stable diamagnetic molecule. The ability of antioxidants to reduce DPPH radicals was determined by a decrease in their 517nm DPPH radical absorbance. The DPPH scavenging ability of the grape seed powder was compared with ascorbic acid a known standard antioxidant. The dosage response curve for the radical scavenging ability is shown in Figures (3)and (5). With rising extract concentration, the scavenging of DPPH radicals increased that is recorded in table 3. The EC_{50} value is a metric generally used to assess the free radical scavenging activity. It is defined as the concentration of the extract necessary for 50 percent scavenging of radicals under the experimental condition used. (CUVELIER et al .1992) A greater antioxidant activity is associated with a lower $EC_{50\ DPPH}$ value. fig.3 shows the $EC_{50\ DPPH}$ value of grape seed extracts based on radical scavenging activities (SADPPH). The EC_{50} DPPH =0.536 mg /ml ,and the IC_{50} of ascorbic acid as a standard antioxidant was estimated as 0.021mg/ml in this work and was significantly different in comparison to grape seed .the scavenging effect of ascorbic acid ranged from 11.44% at concentration 8.25µg/ml to 80.54% at the concentration 66 µg/ml as shown in table 4,fig 4 and 6, while the scavenging effect of grape seed powder is from 2.6 to 49.137at concentration 4.8µg/ml to 1250.µg/ml. The measured absorbance values and calculated percentage inhibition show that the antioxidant activity of the grape seed and standard (Ascorbic acid) is concentration dependent. From the results, the % inhibition of the grape seed exhibited good scavenging ability on DPPH radical which were comparable to ascorbic acid at all concentrations investigated. The biological process behind the antioxidant property is linked to the elimination of free radicals, which affect immune system function and cell signaling. [31] This is especially significant when taking into account grape seed extract's ability to reduce oxidative stress. [32] and decrease low-density lipoprotein (LDL) levels [33] and thereby reduce the inflammatory process related to some diseases. At a result, grape seed powder can be used as a source of natural antioxidant in food technologies.

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Table 3: shows Antioxidant capacity of grape seed powder using DPPH

Concentration(µg/ml)	1250	625	312.5	156.25	78.125	39.0625	19.5312	9.76562	4.8828	Control
OD1	0.508	0.532	0.548	0.574	0.589	0.661	0.698	0.805	0.956	0.992
OD2	0.492	0.538	0.557	0.561	0.612	0.643	0.722	0.789	0.949	0.975
OD3	0.503	0.52	0.55	0.564	0.626	0.652	0.718	0.814	0.971	0.988
Average	0.501	0.53	0.5516	0.5663	0.609	0.652	0.7126	0.8026	0.9586	0.985
RSA% 1	48.426	45.989	44.365	41.725	40.203	32.893	29.137	18.274	2.9441	-0.7106
RSA% 2	50.050	45.380	43.451	43.045	37.868	34.720	26.700	19.898	3.6548	1.0152
RSA% 3	48.934	47.208	44.162	42.741	36.446	33.807	27.106	17.360	1.4213	-0.3045
Average	49.137	46.192	43.993	42.504	38.172	33.807	27.648	18.510	2.6734	0.00
STDEV	0.83	0.9304	0.479	0.6910	1.8966	0.9137	1.305	1.285	1.14	0.9023

Table 4; shows Antioxidant capacity of Ascorbic acid using DPPH

concentration (µg/ml)	66	33	16.5	8.25
	0.171	0.298	0.455	0.785
	0.173	0.298	0.456	0.784
	0.173	0.3	0.453	0.784
Average	0.172333	0.298667	0.454667	0.784333
RSA1	80.69251	66.35303	48.62627	11.3662
RSA2	80.46669	66.35303	48.51336	11.47911
RSA3	80.46669	66.12721	48.85209	11.47911
RSA%	80.54196	66.27776	48.66391	11.44148
STDEV	0.130376	0.130376	0.172472	0.065188

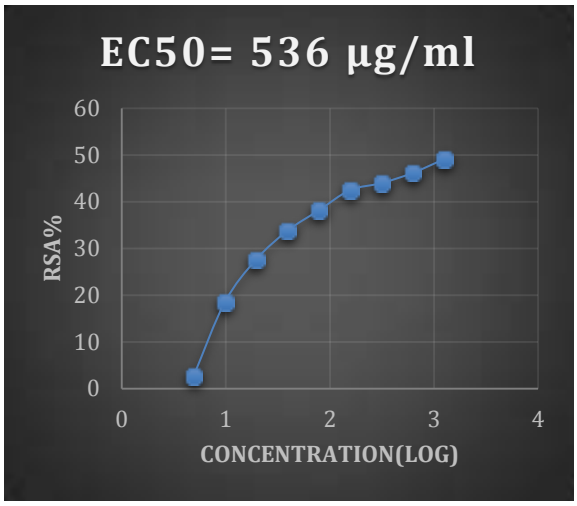

Fig3: demonstrate the percentage of Scavenging activity of grape seed powder on DPPH radicals

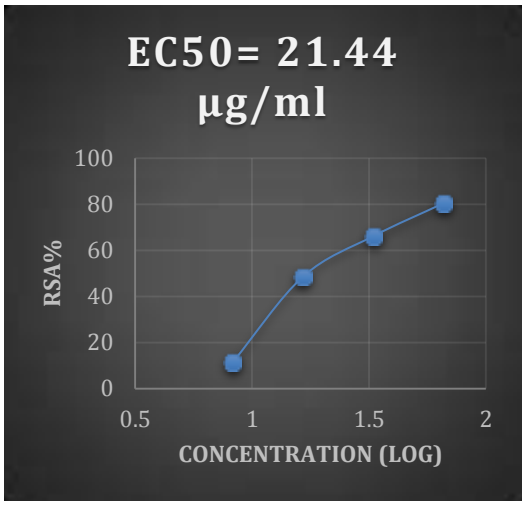

Fig 4: demonstrate the percentage of Scavenging activity of ascorbic acid on DPPH radicals

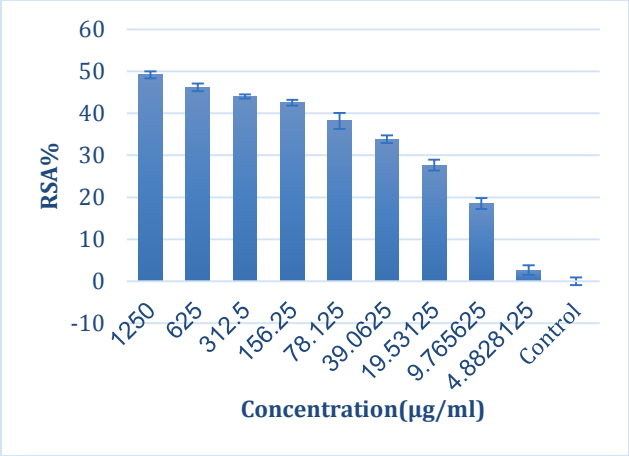

Fig 5: the DPPH radical scavenging activites of grape seed.

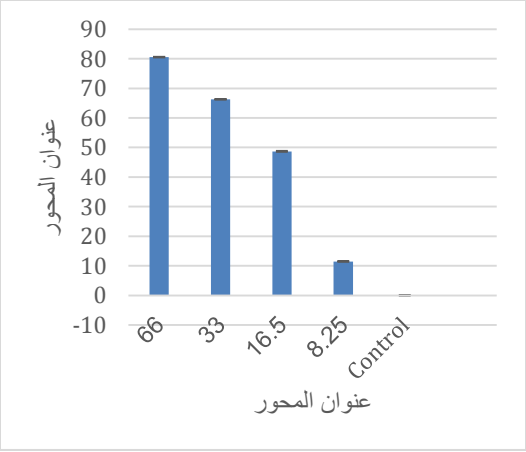

Fig 6: the DPPH radical scavenging activites of Ascorbic acid

3-Determination of mineral contents:

The mineral contents of grape seeds were determined by Inductivity Coupled Plasma Optical Emission Spectrometer (ICP-OES). the minerals composition of seeds is summarized in table (5), fig.7 According to results (Ca,Al,Cu,Fe and Mn)contents grape seed powder was generally found very high respectively.in addition (Cr,Co,Cd) contents of seed were established very low. While Calcium

content was highest (13602.500 µ/L) followed by Al contents was found (6805.402 µ/L) while Cd recorded lowest content(0.0775 µ/L), These variations in the mineral composition of grape seeds could be caused by variations in growth conditions, genetic makeup, harvesting methods, and soil characteristics [34,35]. The main component of bone is calcium, which also aids in the growth of teeth [36]. Since many enzymes need these substances as cofactors, their significance cannot be overstated[37].

Elements	ppb = µ/L
Pb	< LOD
Al	6805.40
Ni	< LOD
Cu	1048.17
Mn	427.116
Fe	1026.046
Co	0.7836
Ca	13602.50
Cd	0.0775
Cr	3.5209
Ba	117.863

Table 5:the mineral contents of grape seed powder

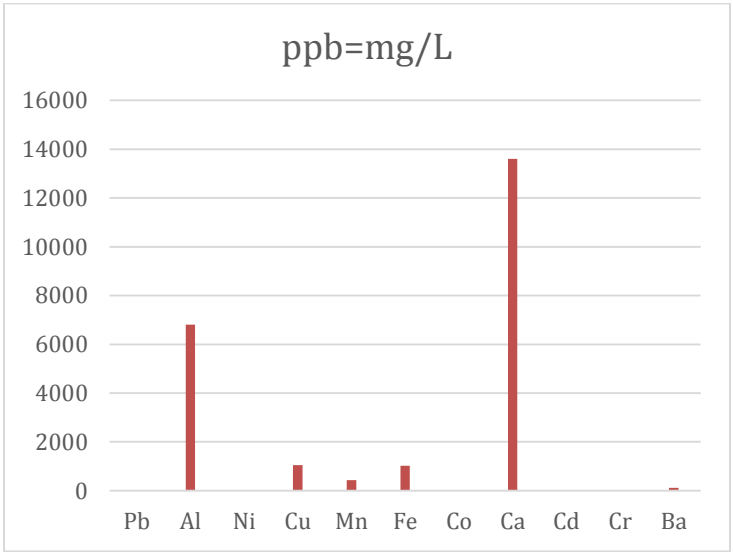

Fig. 7: Graphical representation of elements (ppb=mg/L)

Conclusion:

A significant difficulty is the recovery of value-added compounds from trash and byproducts. The grape seeds' quality characteristics, such as their fatty acid composition, antioxidant activity, and mineral content, were assessed. Analysis of the grape seed powder showed that they contained different major constituents, includes unsaturated fatty acid such as omega-9 and omega 6 respectively, while minor constituents include aldehyde and long chain of hydrocarbons. The findings of this study demonstrated that pomace byproduct, can be used as a dietary supplement. Because humans lack the enzymes necessary to synthesize polyunsaturates, we must obtain our supply of polyunsaturated fatty acids from outside sources. having a high level of linolic acid and oleic acid increased the importance of grape seed in the particular treatment of high cholesterol and atherosclerosis. These major constituents GSP have been reported to possess antioxidant properties. Grape seeds are very powerful antioxidants. Their defense against oxidative harm is one of their potential health advantages. As shown of IC_{50} and DPPH results, it was demonstrated that GSP had greater radical scavenging activity, and can be used as a natural antioxidant activity in food technologies. Most of the chemical compounds identified from grape seed were biologically active compounds and the grape seed exhibited good scavenging ability at all concentrations investigated. GSP could be a source of drug development for oxidative diseases. As a consequence of the study, it can be concluded that grape seeds are healthy sources of both micro and macro minerals and can be used as a dietary ingredient to meet human nutritional needs. It was discovered that grape seeds are significant providers of nutrients and vital components.

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