Grape seeds powder rich in nutrients, bioactive compounds, and antioxidant activities: application in chocolate biscuit

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Abstract:

This study was conducted to find out the effect of use wasted grape seeds powder (GSP) to increase and improve the nutritional value of chocolate biscuit product which added to them. The grape fruits were washed well under running water to remove dust and sediment attached to them, then the seeds were removed from the grapes, they were washed well, drying in shade, ground in a high mixer blender and sieved 750 µm mesh to obtain a GSP 73%. GSP was chemically analyzed, and incorporated into chocolate biscuits in three different levels, 10, 20 and 30% as a potential source of bioactive compounds and natural antioxidants. The results indicated that GSP content was 6.71% moisture, 9.07% protein, 10.52% fat, 31.22% fiber, 2.83% ash and 39.65 % Carbohydrates. GSP was recorded 289.56 Kcal/100g of energy, and chemical analysis indicated that GSP contains high levels of several minerals such as Ca, K and Mg about 701 ,475 and 122 mg/100g respectively. Leucine recorded the higher score 6.62% in GSP, while tryptophane recorded the lowest 2.00%. Phytochemical analysis of GSP of flavonoids, phenols and antioxidant activity in EtOH, MeOH and water extract recorded high scores in EtOH extract 23.89 mg/g, 321.56 mg GAE/g and 243.81 mg/mL respectively. The three different levels chocolate biscuit had a high approval rating, which shows its potential as a good addition to traditional flour.

Key words: *Vitis vinifera L.*, nutritional evaluation, essential amino acid, total phenolics, flavonoids, sensory evaluation

1. Introduction

Grapes (*Vitis vinifera L.*) are some of the world's most popular fruits**.** It is considered one of world’s ancient and significant crops. The oldest evidence for human use dates back 7400-7000 year **(Padilla-González *et al.*, 2022).** They are among the world's greatest fruit crops, producing 68 million tonnes yearly, 38 million of which are processed. The winery and grape juice industries produce approximately 2.5 million tonnes of grape waste each year **(Nowshehri *et al.*, 2015).** Grape processing for ethanol, fruit juice, and wine production generates massive amounts of byproducts such as stems, skin, seeds, and peels **(Tag El-Din *et al.*, 2019).** It has also been lauded as a folk medicinal plant having critical responsibilities in health care and the treatment of a variety of human illnesses **(Habib *et al.*, 2022).** Grapes were ranked second in Egypt, after citrus. Thompson, seedless and Roumy Ahmer of grape varieties account for about two-thirds of the total land area **(Hanaa *et al.*, 2015)**. Grape by-products like marcs, pomace, peels, seeds, and stems have gotten a lot of interest in recent years for their possible health advantages not only as antioxidants, but as anti-obesity, antibacterial, antithrombotic and anti-carcinogenic agents **(Elgaml *et al.*, 2018)**. It a main industrial byproduct of juice manufacturing, possess a high concentration of biologically active elements that are beneficial to health **(Valkov *et al.* 2020)**. It is high in minerals (calcium, sodium, potassium, iron) and vitamins (C, A, B2, B1 and niacin) **(Amin and Edris 2017).** It has 35% fiber, 7% water, 11% protein, 3% minerals, and 7 to 20% fat content. It extracts is abundant in polyphenols, primarily oligomeric proanthocyanins and monomeric flavan-3-ols. It has powerful antioxidant capabilities that act through modifying antioxidant enzyme expression, reducing oxidative damage in cells **(Angeline and Martina, 2019)**. Its oil, often known as grape oil, is extracted of grape seeds. It has higher linoleic acid content than several other carrier oils. When compared to other oil-rich seeds, its oil is mostly composed of triglycerides (TG), which are high in unsaturated fatty acids like as oleic and linoleic acids **(Faris *et al.*, 2016).** Grape seed extracts are high in bioactive polyphenols like flavonoids, which have been shown to have antioxidant, anti-inflammatory, anti-aging, and skin-brightening qualities **(Tao *et al.*,2022).** Grapevine seeds contain flavonoids that have antimicrobial and anxiolytic effects **(Sochorova *et al.*, 2020).** It extracts is a common extract obtained from grape seeds. It is rich in proanthocyanidin oligomers, one of the most important plant flavonoids. These flavonoids have numerous health benefits, such as the potential to improve intracellular vitamin C levels, reduce fragility and capillary permeability and scavenge oxidants and free radicals **(Yildirim *et al.*, 2011)**. Several research have employed grape seed extract to improve the content of beneficial chemicals in yoghurt, frankfurters, and bread **(Elgam *et al.*, 2018).**

The current study sought to ascertain the chemical makeup, nutrients, bioactive components, and antioxidant activity of grape seeds powder. Evaluation of the potential use of GSP in the production of chocolate biscuits.

2. Materials and methods

2.1. Materials

Grape fruits, wheat flour 72% and rest of ingredients were obtained from Spinney’s hypermarket, Minia Governorate, Egypt. Phenolic standards were from Sigmae-Aldrich Chemical Co, Cairo, Egypt. The rest of the chemicals, solvents were of analytical grade and purchased from El-Gomhoria company for trading drug Chemicals and medicals, Cairo, Egypt

2.2. Methods

2.2.1. Preparation of GSP

Grape fruits were washed under running water to remove dust and sediment attached to them, then the seeds were removed from the grapes, washed and drying in shade, then ground in a high blender (Toshiba ElAraby, Benha, Egypt) and sieved in mesh 750 µm to obtain a grape seeds powder 73% and stored at 4C□ until analysis and preparation of the products.

Figure (1): Photo of grape seeds & flour

2.2.2. Preparation of chocolate biscuit

The formula used for chocolate biscuit manufacturing follows the procedure described by **Elwardany and Sheteewy, (2018)**, with minor alterations shown in the Table (1). Butter and sugar were combined to form a creamy mix. The remaining ingredients were then combined to produce the dough, which was smoothed out with a rolling pin to a uniform thickness of 0.6 cm and cut into small hearts before baking in an Ariston oven at 200 C□ for 10 minutes. After cooling, the biscuits were used for sensory evaluation.

Table (1): **Formulation of chocolate biscuit**

Ingredients (g)	WF	GSP	Egg	Butter	Sugar	Salt	Cacao	Vanilla
WF 100%	100	—	10	50	25	2	10	2
W F 90% + GSP 10%	90	10	10	50	25	2	10	2
W F 80% + GSP 20%	80	20	10	50	25	2	10	2
W F 90% + GSP 30%	70	30	10	50	25	2	10	2

2.2.3. Chemical composition of GSP

The chemical properties (moisture, proteins, lipids, ash, and fiber) of GSP samples were determined according to **AOAC, (1995).** Carbohydrates calculated by differences:

Carbohydrates (%) =100- (%moisture +proteins +lipids +ash+ fiber)

2.2.3.1. Minerals analysis

Each tube received 500 mg of GSP placed into a digested glass tube of Kjeldahl digestion unit and 6 ml from the tri-acids mix (nitric acid, perchloric acid, and sulfuric acid in the ratios of 20: 4: 1 v/v). The tubes' components were gradually digested as follows: 30 minutes at 70, 30 minutes at 180, and 30 minutes at 220. After digestion, the liquid was chilled, dissolved in distilled water, and the volume in a volumetric beaker was raised to 50 ml. Following filtration in ashless filter paper, aliquots were tested for selenium using an atomic absorption spectrophotometer, model 2380 Perkin-Elmer **(Singh *et al.*, 1991)**.

2.2.3.2 Amino acids

Amino acids were analyzed in HPLC according to the method of **Lindroth and Mopper (1979).** The results were expressed in mg per 100 g sample d.w.

2.2.3.3. Total phenolics and flavonoids

On such an orbital shaker calibrated to 200 rpm, 200 mg of GSP was extracted during a period of two hours at room temperature with 2 mL of 80% EtOH/ MeOH/ H2O containing 1% hydrochloric acid. After centrifuging the mixture at 1000g for fifteen minutes, the supernatant was decanted into 4 mL vials. The pellets were mixed and the total phenolics analysis was performed. One 100 microliters of the acquired extract were blended with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold to distilled water) and let stand at 22 0C for five minutes before adding 0.75 mL of sodium bicarbonate (60 g/L) solution and measuring absorbance at 725 nm after 90 minutes at 22 0C. The results are given in terms of GA and equivalents **(Singleton and Rossi 1965)**. 150 L of sodium nitrate (5%) and 2.5 mL for distilled water were put to an aliquot (0.05 mL) of the extract/standard (Quercetin equivalents, QE). 0.3 mL of aluminum chloride (AlCl3, 10%) has been added after 5 minutes. After 6 minutes, 1 mL of NaOH (0.001 M) and 0.55 mL from distilled water were mixed into the mixture, which was then kept at room temperature for fifteen minutes. The mixes' absorbance was determined using a 510 nm spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan). Extract samples were tested at final condensation of 0.1 and 0.15 mg/mL. Total flavonoid content was calculated using the QE standard curve methodology and reported as catechin equivalent. **(Zhishen *et al.*, 1999).**

2.2.3.4. Antioxidant activity and DPPH scavenging

In round-bottom flasks (50 mL) having 0.02 mL containing linoleic acid and 0.2 mL of Tween 20, 1 mL solution and 0.2 mg/mL in chloroform, was added. Following that, each mix was dosed using 0.2 mL of 80% MeOH (as a control) or the relevant sample extract or standard. After drying under vacuum in room temperature, 50 ml of oxygenated distilled water had been added and the mixture was agitated to generate a liposome solution. The samples were then thermally autoxidized for 2 hours at 50 0C. The absorbance for the solution at 470 nm was measured on a spectrophotometer at ten-minute intervals, and the rate bleaching was determined by fitting a linear regression onto data over time. Every sample was tested in triplicate. The control was a concentration of -tocopherol in 80% methanol. The following equation was used to quantify antioxidant activity (AA) as a percentage inhibition relative to the control **(Al-Saikhan *et al.*, 1995)**. AA= ($R_{control}$ - $R_{specimen}$) / $R_{control}$ x 100, while $R_{control}$ and $R_{specimen}$ were the bleaching values for beta-carotene in reactant mixes with sample extract and without antioxidant, respectively **(Marco, 1968).** 2.4 mL for 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM through methanol) has been combined mixed 1.6 mL of sample extract at various concentrations to make a solution. The reaction mix was thoroughly vortexed and allowed to sit for thirty minutes at room temperature. The absorbance of the combination was determined spectrophotometrically at 517 nm (UV-160A, Shimadzu Corporation, Kyoto, Japan). BHT was used as a reference. The following equation was used to compute the percentage scavenging of DPPH radicals' activity. (DPPH) radical scavenging activity (%) while A0 represents the absorbance of a control and A1 represents the absorbance of the specimen/BHT. The graph was then used to calculate the IC50 by plotting inhibition (%) versus concentration **(Desmarchelier *et al.*, 1997).**

2.2.4 .Evaluation of sensory properties of chocolate biscuit

Sensory evaluation was carried out using 20 panel tests at Minia University, Home Economics Department, Faculty of Specific Education. The items were coded with a number prior to testing, and each panel test got four chocolate biscuit samples in a sealed bag coded with a distinct number. Using a 1-10 scale, each participant evaluated the goods' taste, odor, color, texture, and overall approval.

2.2.5. Ethical approval

The Scientific Research Ethics Committee (SREC) Faculty of Specific Education, Minia University, Minia, Egypt, approved all experiments for this study, particularly the sensory evaluation ones.

2.2.6. Statistical analyses

Data were analyzed with GLM (General le\Linear Model) program using statistical analysis system (**SAS, 2003**). Mean values were compared by Duncan's Multiple Test.

3.Results and discussions

3.1. Chemical composition of GSP

The proximate analysis of GSP is shown in Table (2). Results showed that fat and carbohydrates content was found to be high in grape seeds (GS) (10.52 and 39.65) respectively. oils in GS are contained monounsaturated fatty acids and polyunsaturated fatty acids in higher levels than saturated fatty acid, also oil is rich in α, β and γ-tocopherols and α and γ-tocotrienols, which are consider as natural fat-soluble antioxidants which benefits for human nutrition **Rodríguez and Ruiz (2016)**. The results in the present study are in agreement with that confirmed by **Ahmed ,(2012)** reported that fat and carbohydrate content of white GS was 10.38 and 37.25% respectively. Also results showed that GS contented 6.71, 9.07, 31.22 and 2.83% for moisture, protein, fiber, and ash respectively, which in agree with **Mironeasa *et al*., (2015)** mentioned that the content of grape seed from moisture, protein and ash were 7.16, 9.78 and 3.35 respectively, and (**Gupta *et al*.,**

2019 ; Hamdi and Ahmed,2021) reported that grape seeds flour contains a high percentage of fiber 42.98% , moisture was7.16% and ash reached 2.36% .On other side the result doesn't agree with **Oprea *et al*., (2022**) report that the percentage of protein in grape seed flour was 16.32% , raw fiber was 83.01%. **Nardoia *et al*., (2017**) indicated that protein and fiber content was 19.64% and 25.28 respectively.

Table (2): Chemical composition (g.100g-1) of GSP

Parameters	GSP
Moisture	6.71 ± 0.37
Total protein	9.07 ± 0.16
fat	10.52 ± 0.39
fiber	31.22 ± 0.91
Ash	2.83 ± 0.09
Carbohydrates	39.65 ± 1.13

Each value represents the mean ±SD of three replicates.

3.2. Nutritional evaluation of GSP

The nutritional evaluation of GSP was investigated in Table (2). GSP was recorded (289.56 Kcal/100 g and 9.07 g) of energy and protein respectively. **Özcan *et al*., (2012)** reported that energy values of GS were established to be between 102.28 :148.07 kcal g. The results confirmed that the protein content in each 100 g of GSP was 9.07g which equals nearly 15% of the RDA for adults.

Data in Table (3) observed that the grams daily of energy that required obtaining RDA for GSP was 1001.41 g. The consumption of 100 g from GSP will cover 9.98 of RDA of energy, while taking the same amount of GSP will almost cover 14.39 of RDA of adult in protein.

Table (3): Nutritional evaluation of GSP

Nutritional evaluation	RDA (1989)	GSP
Energy (Kcal/100g)	**2900 Kcal**	**289.56 ± 2.59**
***G.D.R. (g)**		**1001.41 ± 8.08**
****P.S./100 g**		**9.98 ± 0.09**
Total protein	**63 g**	**9.07 ± 0.20**
G.D.R. (g)		**695.07 ± 15.06**
P.S./ 100 g		**14.39 ± 0.31**
Dry matter (%)		**93.29 ± 0.46**

* G.D.R. (g): Grams consumed to cover the recommended daily allowance of adult man according to RDA (1989). ** P.S. /100 (%): Percent satisfaction of RDA of adult man when consuming 100g of GSP. Each value represents the mean of three replicates ±SD.

3.3. Minerals composition of GSP

Some mineral elements in the GSP samples were showed in Table (4). The results indicated that GSP contains higher amount of Ca and K (701 and 475 mg) and it contain a moderate amount of P and Mg (157 and 122 mg) while a low amount of Na and Zn were present in grape seeds (13.56 mg and 1.49 mg) respectively. Our results were agreeing with **Sanchez *et al*., (2022)** report that GS contains high amount of Ca and low amount in Zn, and nearly with **Amariei *et al*., (2017)** reported that the minerals in GSF from calcium, zinc and sodium were 405.89, 0.73 and 296.98 mg/100g respectively. Because of a large amount of calcium, potassium and phosphorus content in GSP **Valková *et al*., (2021)**, and also the essential elements such as iron, manganese, copper and zinc **(Felhi *et al*., 2016**; **Al Juhaimi *et al*., 2017**), therefore, grape seeds could be considered an excellent source of bio elements and dietary supplement (**Jiang *et al*., 2022)**.

Optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could reduce individual risk factors, including those related to cardiovascular disease **Mironeasa *et al*., (2015)**. Animal studies have found that combination of GS extracts to a low calcium diet can increase density and strength of bone **Ishikawa *et al*., (2005)**, and may decreases destruction of bones in inflammatory autoimmune arthritis (**Park *et al*., 2012).**

Table (4): Mineral composition of GSP compared with RDA (1989)

Element	Mg/100g	RDA (mg/day) **	N.F%*
Mg	122 ± 7.35	350	34.85
K	475 ± 12.04	1950	24.36
Ca	701 ± 22.04	800	87.63
Na	13.56 ± 0.49	500	2.71
P	157 ± 5.44	800	19.63
Zn	1.49 ± 0.03	15	9.93

Each value represents the mean ±SD of three replicates.

*N.F. Nutritional Factor **RDA = Recommended Dietary Allowances (1989)

3.4. Amino acids composition of grape seeds powder

The amino acids content of GSP are shown in Table (5). The major indispensable amino acids present in GSP were leucine 6.62% recorded a high score followed by valine, threonine, lysine and isoleucine 5.10%, 4.86%, 4.1 0% and 2.88% respectively. while tryptophane recorded low score 2.00% compared to their other indispensable amino acids. On the other hand, the results in Table (5) reveal that non-essential amino acid GSP were detected as high content in glutamic and glycine 13.15% and 8.40% respectively. The GSP had moderate amounts of aspartic, alanine, arginine, proline and serine 5.30%, 4.65%, 4.20%, 4.10% and 3.32% respectively. While histidine and cysteine recorded the lowest 1.90% and 1.60% respectively. However, the total essential amino acid and total non-essential amino acid 31.31and 61.20 respectively.

Such data are in accordance with **Varedesara *et al*., (2021**) confirmed that isoleucine acid in GS was 2.98 %, serine acid was 3.94 %. And nearly with **Costa *et al*., (2022)** indicated that grape seed had 5.6% from alanine, cystine 2.5%, glycine 10.2%, isoleucine 3.1%, lysine 4.5% and threonine 4.0%. But disagreed with **Bastante *et al*., (2022)** showed that the amino acids content of defatted grape seed meal was 2.50% for threonine acid, serine ranged from 6.98% to 11.34%, alanine from 7.83% to 11.06%, valine from 5.46% to 7.53% and arginine from 7.84% to 9.79%.

Table (5): Amino acid composition of GSP

Amino acid	g.100g^{-1}	g/16N%
Isoleucine	0.26 ± 0.02	2.88
Leucine	0.60 ± 0.05	6.62
Lysine	0.37 ± 0.01	4.1 0
Methionine	0.19 ± 0.003	2.10
Phenylalanine	0.33 ± 0.01	3.65
Threonine	0.44 ± 0.01	4.86
Tryptophane	0.18 ± 0.01	2.00
Valine	0.46 ± 0.01	5.10
Total (E.A.A) **		31.31
S-CM cystein	0.14 ± 0.005	1.60
Arginine	0.38 ± 0.009	4.20
Histidine	0.17 ± 0.004	1.90
Tyrosine	0.28 ± 0.006	3.10
Serine	0.30 ± 0.007	3.32
Glutamine	0.28 ± 0.007	3.10
Asparatic	0.48 ± 0.01	5.30
Gylcine	0.76 ± 0.02	8.40
Alanine	0.42 ± 0.02	4.65
Proline	0.37 ± 0.01	4.10
Cysteic	0.19 ± 0.01	2.10
Glutamic	1.19 ± 0.03	13.15
Asparagin	0.28 ± 0.006	3.10
Ornithine	0.27 ± 0.01	3.00
Total (N.E.A.A)***		61.20

*= Each value represents the mean ±SD of three replicates.
**E.A.A: Essential Amino acid
*** N.E.A.A: Non-Essential Amino acid

3.4.1. Essential amino acid composition of GSP

Data in Table (6) showed the amino acid score of GSP, the percentages of all eight essential amino acids or amino acid pairs in the defatted. GSP exceeded the percentages of these amino acids in the WHO/FAO standard. Noteworthy the major essential amino acid of GSP were tryptophan, methionine + cystine, valine, threonine and phenylalanine + tyrosine (181, 148, 145, 143 and 107%) respectively followed by isoleucine and leucine (103 and 100%) and respectively. On the other hand, it is very poor in lysine 71%. The essential amino acid content of grape seeds powder is in a good amount as recommended by FAO / WHO (1989).

Data in the present study reveal that GSP is considered as a good source of essential amino acids. Therefore, it could be used as a food additive to bakery and meat products to improve its qualities.

Table (6): Essential amino acid composition of GSP

Amino acid	FAO /WHO ideal protein *	%of total amino acid	Amino acid /ideal×100
Isoleucine	**2.8**	**2.88**	**103**
Leucine	**6.6**	**6.62**	**100**
Lysine	**5.8**	**4.10**	**71**
Methionine + Cystine	**2.5**	**3.70**	**148**
Phenylalanine+ Tyrosine	**6.3**	**6.75**	**107**
Threonine	**3.4**	**4.86**	**143**
Tryptophan	**1.1**	**2.00**	**181**
Valine	**3.5**	**5.10**	**145**

* FAO/ WHO (1989).

3.5. Total phenolics and flavonoids

In the present study, the total phenol and flavonoid content of grape seeds powder were represented in Table (7). The study has reported that the total phenolic compounds in various extracts of grape seed powder ranged from 151 - 321 mg GAE/g. EtOH extract showed the highest total phenolic content (321 mg/g), while H2O extract had the lowest value (151 mg/g). The phenolic content in various solvents decreases in the order of EtOH > MeOH > water. In our study, the total flavonoid content in various extracts of grape seed powder EtOH extract of GSP contain a high amount of 23 mg/g and H2O extract had the lowest value11.53 of flavonoids.

The overall trend was the same as reported by **Hanaa *et al.*, (2015)** found that extraction with methanol extract led to a high phenolic content of 372.5 mg GAE/g, while water extract gave the lowest phenolic content 186 mg GAE/g. Our result was near to that **Yarovaya and Khunkitti., (2019)** who reported that the total phenolic content contained in 1 mg/mL of GSE was equivalent to 205.1±14.024 mg GAE/g. and **Alfaia *et al*., (2022)** found that the total phenols content in grape seed was 261.3 mg GAE/g. While total phenolic content in seed flour of grape fruit was 542.8 g/kg **(Acun and Gül ,2014)**. On another hand GS had a high total phenolic compounds content (3681.6mg\100mg) **(El-Demery and El- Refai, 2015)**. **Habib *et al*., (2022)** indicated that total phenolics of GSE was evaluated for their total phenolic content which was 14.234 ± 20.34 mg/100 g and total flavonoids were 6721 ± 14.21 mg/100 g. Also, total phenolic and total flavonoids the GSP is 46.44 mg GAE/ g and 4.98 mg QU respectively **(EL-Beltagi *et al*., 2016).** Total phenolic content was found in GS (9.75 mg/g DW), followed by grape leaves (7.32 mg/g DW), while total flavonoids content in GS(4.88 mg/g DW) followed by grape leaves(2.86 mg/g DW) **(Abdel‑Khalek and Mattar , 2022).**

The phenolic compounds have great potential due to their antioxidant capacity and health benefits to prevent coronary problems and other chronic diseases, diabetes, or neurodegenerative issues (**Cotea *et al*.,2018)**. Some food components such as dietary fiber and polyphenolics that reach the gut may prevent or moderate weight gain, abdominal adipose weight increases in blood lipids, fatty liver **(Arvik *et al*.,2018)**. Grape seed polyphenols have been reported to exhibit a lot of biological properties. Resveratrol had cancer chemo preventive activity in assays representing three major stages of carcinogenesis **(Zhu *et al*., 2015).**

Table (7): Total Phenolics and Flavonoids of GSP

Phytochemical analysis	Different extractions media		
	EtOH extract	MeOH extract	H2O Extract
Total phenolics (mg GAE/g, d.b.)	321.56 ±10.35	296.78 ±7.15	151.78 ± 5.3
Flavonoids (mg QE/g, d.b.)	23.89± 1.36	17.95 ±0.95	11.53 ± 0.65

GAE /g of dry seed extract; b: mg QE/g of dry seed extract.
.Each value represents the mean ±SD of three replicates

3.6. Antioxidant activity of GSP

Table (8) shows the free radical scavenging activity and antioxidant activities for all tested grape seed powder extracts. AA recorded a high value of EtOH extract 243.81% mg/mL and a low score in H2O extract 89.83% mg/mL . Also, the GSP recorded the lowest score of DPPH scavenging from H2O Extract 0.191 mg/ml DPPH and the high score in EtOH extract 0.453 mg/ml DPPH. While MeOH extract recorded 142.53 % and 0.429 mg/ml for AA and DPPH respectively. Data has confirmed that the high antioxidant capacity of all extracts has been observed and related to the presence of polyphenolic compounds with good antioxidant activity.

The result is similar to **Gougoulias and Mashev,(2008)** reported that phenols in GS ranged from 72.2 to 104.8 µmol DPPH/g DM. And the antioxidant activity ranged from 618.2 to 805.0 µmol for grape seeds varieties. Our results agree with **Hanaa *et al*., (2015) and Aldubayan, (2018)** showed that the highest DPPH scavenging activities by ethanol extract of grape seed powder and the lowest DPPH scavenging activities were shown by water extract of grape seed powder.

Additionally, several reports found that GSE showed higher scavenging activity than all other extracts with EC50 0.259µg extract/µg DPPH **(Ishmael *et al*., 2012)**. The antioxidant capacity of GSP at methanol, ethanol and water extract were 664.2 , 673.5

and 88.6 µM TE /g dry matter respectively**(Li *et al.*,2008)**. **Kwatra, (2020)** reported that the antioxidant capacity of grape seed extract using DPPH ranged between 17 and 92 mmol antioxidant equivalent (TE)/g. **Wongnarat and Srihanam, (2017)** noticed that antioxidant activities of the GS was 55.96 µg/mL by DPPH but 6743 g/mL of trolox equivalents p≤0.05 by the DPPH assay **(Li *et al.*,2008)** .

Antioxidant activities of GS extracts ranged from 66.41% to 81.40% **(Rababah *et al.*, 2008). Jassy *et al.*, (2020)** examined the antioxidant activity of different varieties of grape seed by DPPH assay he found at concentrations of 50%, 60% and 70% were ranged from 53.69 to 76.57 respectively. DPPH of different tissues in two cultivars of grape seed 108.5 and 109.1 µmol TE/g FW **(Shen *et al.*,2020)**.

The use of GSE has gained ground as a nutritional supplement in view of its antioxidant activity. which can be used as dietary supplements, or in the production of phytochemicals, providing an important economic advantage **(El Gengaihi and Abou Baker ,2017).** Grape seeds had the highest antioxidant activity it has protection against oxidation **(El-Bastawesy *et al.*, 2007).** Grape phenolics, including flavonoids and related polyphenols from grapes, grape fruit, and grape seeds have generated remarkable interest based on positive reports of their antioxidant properties and ability to serve as free radical scavengers **(Çetin and Saodyç, 2009).**

Table (8): Antioxidant activity of GSP

Antioxidant capacity	Different extractions media		
	EtOH extract	MeOH extract	H2O Extract
AA (%), (α-tocopherol, 50 mg/mL)	243.81± 11.32	142.53± 5.22	89.83± 1.75
DPPH scavenging (IC50, mg/ml)	0.453± 0.04	0.429± 0.04	0.191± 0.01

Each value represents the mean ±SD of three replicates

Sensory evaluation of Chocolate biscuit samples:

Sensory characteristics play a significant role in accepting or refusing various products and deriving satisfaction from their use. Color, odor, taste, and general approval were used to determine sensory analysis in this study (**Varedesara *et al*., 2021)**. As per statistical analysis results it can be seen in Table (9), as with the sensory evaluation of chocolate biscuits which was produced by adding GSP achieved fewer points than the control group.

Color is an important attribute of food choice and acceptance, for the value of color, samples prepared by GSP 20% and 30% had the highest color value (9.25 and 9.25) respectively compared with samples prepared by GSP 10 % which had less value in terms of color (8.65), that is where compared to controls (9.3).

Without good taste, the product may be acceptable, but it is potentially unacceptable. The sensory qualities of food products, the taste is an important factor to consider **(Muhimbula *et al*., 2011).** For the score of taste, there was in difference between the control sample (WF) and other samples prepared with GSP. The samples GSP 10% and 30% had taste values (8.98 and 8.7%) respectively, while the sample prepared by GSP 20% had the lowest value of taste 8.5%. The samples with GSP 10% were more perfect than the samples with GSP 20% and 30%. Texture a basic acceptability element of foods, has entered the consumer purchase decision Chocolate biscuit prepared by GSP 20% recorded the highest score 9.25 of Texture. While Chocolate biscuits fortified with 10% and 20% from GSP recorded high values 9.25 and 9.25 of odor and fortified with 30% GSP recorded the lowest value 9.05of odor. The samples with 15 and 20% GSP were more perfect than the samples with 30% in Overall acceptability compared to the control sample (WF).

Our result is in accordance with study by **Brykova *et al*., (2017)** who stated that adding grape seed and grape skin powders leads to the improvement of the organoleptic (external appearance, taste, and smell) and the physicochemical (specific volume, wetting ability, form stability) characteristics of the quality of butter biscuits. And nearly with **Maman and Yu, (2019)** who found that the control cookie had the highest scores in all the sensory attributes and overall liking, followed by the cookies with 2.5% of 209 µm GSF. The cookies with 5% GSF of 104 µm received the lowest scores. Cookies with 5% SF additive were preferred more than the control group and cookies with 7.5% grape seed flour additive were ranked in the same group as the control group **(Acun and Gül, 2014).** In spite of **Valková *et al*., (2021**) decided that moreover, data from physical evaluation indicated no considerable impact of grape (*Vitis vinifera L.)* seed volume of bread loaves, hardness of the bread crumb and crust.

Table (9): Sensory evaluation (degree) of chocolate biscuit samples

Sensory properties / Samples	Color	Texture	Odor	Taste	Overall acceptability
WF 100%	9.3 ± 1.13[a]	9.55±0.69 [a]	9.45±0.86 [a]	9.35± 0.96 [a]	9.25±0.98 [a]
WF 90% + G S P 10%	8.65 ±1.15 [a]	8.9± 1.12 [a]	9.25± 0.92 [a]	8.98±1.43 [a]	8.9±1.49 [a]
WF 80% + G S P 20%	9.25 ± 0.85 [a]	9.25± 0.91 [a]	9.25±0.89 [a]	8.5±1.16 [a]	8.9±1.02 [a]
WF 70% + G S P 30%	9.25 ± 0.91 [a]	8.95±1.31 [a]	9.05± 0.82 [a]	8.7±1.42 [a]	8.85±1.15 [a]

Each value represents the mean of twenty replicates ±SD. Mean values with the different letters in the same column mean significantly different at $p \leq 0.05$.

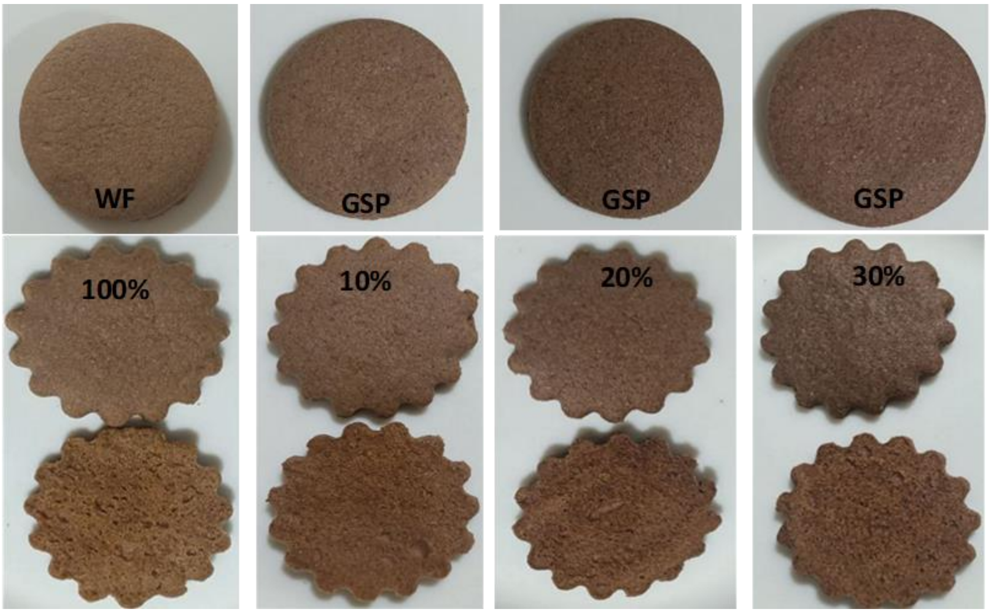

Figure (2): Photo of chocolate biscuit

Conclusion

This study found that grape seeds powder has a high nutritional value due to its content of protein, fat, fiber, and carbohydrate. In addition to, minerals and amino acids. Grape seeds are a good source of bioactive compounds such as flavonoids, polyphenols, and antioxidant activity, which had important for human health. It could be concluded that fortified wheat flour with GSP led to improve the nutritional quality of chocolate biscuit products. Therefore, we recommend using GSP in our daily diets for its health benefits.

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مسحوق بذور العنب الغني بالمغذيات والمركبات النشطة بيولوجيا وأنشطة مضادات الأكسدة : التطبيق بسكويت الشوكولاته

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المستخلص

أجريت هذه الدراسة لمعرفة تأثير استخدام مسحوق بذور العنب في زيادة وتحسين القيمة الغذائية لمنتج بسكويت الشوكولاتة المضافة إليه . تم غسل ثمار العنب جيداً تحت الماء الجاري لإزالة الأتربة والرواسب الملتصقة بها ، ثم تم إزالة البذور من العنب ، وغسلها جيداً ، وتجفيفها في الظل ، وطحنها في خلاط عالي الجودة ، ونخلها للحصول على مسحوق بنسبة استخلاص 73٪. تم تحليل المسحوق الناتج كيميائيًا ، ثم خلطة لانتاج بسكويت الشوكولاتة بثلاثة خلطات مختلفة ، 10 و 20 و 30 ٪ كمصدر محتمل للمركبات النشطة بيولوجيًا ومضادات الأكسدة الطبيعية. أشارت النتائج إلى أن مسحوق بذور العنب يحتوى على 6.71٪ رطوبة ، 9.07٪ بروتين ، 10.52٪ دهون ، 31.22٪ ألياف ، 2.83٪ رماد ، 39.65٪ كربوهيدرات ، كما سجل محتوى عالى من الطاقة 289.56 سعر حرارى / 100 جم. كما اظهر التحليل الكيميائي احتواء المسحوق على مستويات عالية من العديد من المعادن مثل الكالسيوم والبوتاسيوم والمغنسيوم 701 و 475 و 122 مجم / 100 جم على التوالي. وسجل الحمض الامينى الاساسى الليوسين اعلى نسبة 6.62 ٪ بينما سجل التربتوفان اقل درجة 2.00٪. وكانت أعلي نسبة للفلافونيدات والفينولات والنشاط المضاد للأكسدة في مسحوق بذور العنب في المستخلصات المختلفة من في الإيثانول و الميثانول و الماء في مستخلص الأيثانول 23.89 ملجم/ 100جرام و 321.56 ملجم حامض الجاليك المكافئ /100جرام و 243.81 ملجم / مللتر على التوالي. وحصل بسكويت الشوكولاتة المضاف اليه نسبة من مسحوق بذور العنب على نسبة عالية من القبول ، مما يدل على إمكانيته كإضافة جيدة للدقيق التقليدي.

الكلمات المفتاحية : *Vitis vinifera L* ، القيمة الغذائيه ، الأحماض الأمينية الأساسية ، الفينولات الكلية ، الفلافونويد ، التقييم الحسي