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The effect of using ultrasound pretreatment and pectinase enzyme on the extraction efficiency and antioxidant properties of the polyphenolic extract of *sour grape (Vitis viniferia)* waste

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ABSTRACT

Today, the extraction and use of biologically active compounds from agricultural and food wastes has received much attention. In the present research, the effect of enzyme treatment, ultrasound and the combined effect of enzyme treatment and ultrasound on the extraction of phenolic compounds of sour grape wastes was done so that 2 pectinase enzymes (Pectinex Ultracolor and Pectinex Yildamesh) at levels of 10, 20 and 30 mg/kg and ultrasound (times 10, 25 and 40 minutes) and (sound intensity 30, 60 and 90%) were used. The effect of enzyme treatment, ultrasound and the combined effect of enzyme treatment and ultrasound were investigated. According to the obtained results, the highest extraction efficiency belonged to Yaldemesh enzyme and with increasing sound intensity, the extraction efficiency increased significantly. On the other hand, increasing the extraction time led to an increase in extraction efficiency ($p\leq0.01$). So that the highest extraction efficiency was observed in the sample extracted by Yaldemesh enzyme and under ultrasound at 90% sound intensity for 40 minutes ($p\leq0.01$). According to the obtained results, the highest flavonoid, total phenol and antioxidant activity (DPPH, FRAP, ABTS) belonged to Yaldemesh enzyme, and with increasing sound intensity, antioxidant activity increased significantly ($p\leq0.01$). On the other hand, increasing the extraction time led to a significant increase in antioxidant activity ($p\leq0.01$). The highest antioxidant activity belonged to the sample extracted by Yaldemesh enzyme and subjected to ultrasound at a ultrasound intensity of 90% for 40 minutes and was introduced as the best treatment.

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1- Introduction

According to the reports of food waste of fruits and vegetables, it is estimated that between 20 and 40% in the primary agricultural products, which continues during the production stages and until it reaches the final consumer [1]. A wide range of by-products of fruit and vegetable processing, especially in the juice production industry, includes leaves, peels, pulp, a large amount of which remains unused [2]. Bioactive compounds extracted from food-agricultural waste can be used as sources of antioxidant compounds or used in the development of useful products [3]. Grape pomace mainly consists of grape skin, seeds, stem and remaining pomace [4]. Approximately 9 million tons of these wastes are produced annually in the world, which is about 20 percent of the total weight of grapes used in the production of fruit juice and wine [5]. In the case of phenolic compounds, the average of lignin is reported to be 17-24% by weight [6]. Condensed tannins (proanthocyanidins) are another main group of polyphenols found in the pulp, along with other small phenolic compounds that have high health benefits such as cardioprotective, neuroprotective, anti-inflammatory, anticancer and antimicrobial activities. Among them, the most abundant phenolic acids (caffeic, gallic, protocatechuic, 4-hydroxybenzoic and syringic acid), hydroxytyrosol and flavonoids are mainly derivatives of catechin and epicatechin as well as anthocyanins [4]. Extraction of polyphenols from food/pharmaceutical waste with the help of enzyme(EAE) It has also been reported that based on the ability of enzymes such as cellulase,b-Glucosidase, xylanase, beta-gluconase and pectinase are based on destroying the structure of the cell wall and polymerizing plant cell wall polysaccharides, which leads to the release of bound compounds. The feature of enzymes in increasing the biological activity of extracts is determined by the hydrolysis of compounds with high molecular weight to lower molecular weight. Using water as a solventEAE Instead of organic chemicals,EAE turns it into an environmentally friendly technology in the extraction of bioactive compounds. ApplicationsEAE It has been reported in the extraction of polyphenolic compounds from grape waste, green pistachio skin and pomegranate skin.Pectinex is a mixture of several types of pectinase, which is mainly designed for the treatment of fruit and vegetable pulp and the maceration of plant tissues. Pectinex by a selected strain of fungi*Aspergillus niger* it is produced. Among some commercial enzyme products, Pectinex Yildemesh enzymes andPectinex UltracolorThey are known for their high pectinase activity. Pectinex yield It is an enzyme that is used both in the first crushing and in the pulp crushing process, and it selectively decomposes pectin to reduce viscosity. During the first crushing, it significantly increases the yield in fruit juice processing. In the operation of crushing the second pomace, it has the highest yield of fruit juice. Pectinex Ultracolor is used for crushing and dehydrating without the use of color-destroying glycosidases, and as a result, the color is more intense and color stability is improved. It also increases the efficiency of juice and better filtration [7]. Ultrasound extraction technology is widely used in the extraction of oil, polysaccharides, proteins and flavonoids from plants. Research shows that this technology has high efficiency and low energy consumption and prevents the

destruction of effective compounds [8]. The systems that work with these waves are bath and probe, which can be used in laboratory and industrial scale [9]. Ultrasonic waves cause more diffusion of solvent in cellular materials and therefore improve mass transfer and cell wall disruption to facilitate the release of bioactive components [10]. Ultrasonic waves prevent the increase in temperature and thermal destruction of bioactive compounds. Also, it has an effective role in reducing extraction time, using lower amounts of solvent, reducing process costs and benefiting from high level automation. Ultrasound has been used in the extraction of polyphenols from the wastes of the wine industry, such as the extraction of anthocyanins from red grape pomace and wine, trans-resveratrol from red grape pomace, and polyphenols from red grape pomace [11]. Ultrasound has been reported in the recovery of polyphenols from apple, citrus, tomato and onion wastes, olive wastes, potato skin wastes, carrot pulp (black), wheat bran and mung husk [13,12]. The main mechanism of ultrasound extraction is related to the phenomenon of cavitation, during which very small bubbles are formed in the liquid mass and quickly grow to a critical size and then explode. The explosion of these bubbles is often associated with the release of high amounts of energy which is applied to the surrounding environment in the form of shear stress [14]. It is worth mentioning that the studies conducted by different researchers have made it clear that the explosion of bubbles in the vicinity of solid particles is asymmetric, so that it causes a flow of liquid to be pulled towards the surface of the particles at a very high speed. The impact of these microjets on the surface leads to wear, breakage and destruction [15]. Considering the significant amounts of total phenol content in agricultural wastes such as waste and the effective role of pretreatments such as ultrasound or the use of enzymes in the extraction of the mentioned compounds, in this research the effect of using ultrasound pretreatment and pectinase enzyme on the extraction efficiency and antioxidant properties of polyphenolic extract Ghor wastes*(wine vines)* was investigated.

2- Materials and methods

2-1 Materials and equipment

The best *(wine vines)*It was obtained from the factories producing Abghoreh. All chemicals used for chemical analysis were from Merck, Germany. devices usedHPLC (Zwick، Germany, under vacuumMemmert، Germany), spectrophotometer (), ultrasound (Ultrasonic cleaner, baker, China), rotary evaporator under vacuum(EYELA, Japan), pH Meter (pHMeter Hana model penHanna HI98127, China) Was.

2-2 Methods

2-2-1 Preparation of slag

The scum was collected immediately after the dewatering process. These wastes were kept frozen until drying. The slags were dried at room temperature for approximately 48 hours until their moisture content reached 10%. Then the slag was crushed and sieved to select particles with dimensions between 0.6 and 1 mm [16].

2-2-2 Extraction of polyphenolic extract

2-2-2-1 extraction with enzyme

Ghor pomace was exposed to three levels (10, 20 and 30 mg/kg) of two types of pectinase enzymes (Pectinex Ultracolor and Pectinex Yildemesh, Novozims, Denmark). It should be noted that considering the activity of enzymes, their specific concentration was used. Announced Pectinex Ultracalor enzyme Chest/ml10000and the declared activity of pectinex enzymePEU/g7/3 Is. In order to increase the effectiveness of the enzymes, at the optimal

temperature of the enzymes activity (55 degrees Celsius) in different times of 40, 60 minutes, the greenhouse was done. After placing the samples in the greenhouse, they are placed at 80 degrees Celsius for 5 minutes to deactivate the pectinase enzyme. Then, the extract was filtered under vacuum and the extract was stored in dark glass containers in the refrigerator at 5 degrees Celsius to be used for the experiments. The filtered extract was first concentrated by a rotary evaporator under vacuum at a temperature of 40 degrees Celsius and the concentrated extract was poured into 15 cm plates and dried in a vacuum oven at a temperature of 45 degrees Celsius until reaching a constant weight. The dried extract was scraped from the bottom of the plates and stored in McCarthy jars with lids protected by aluminum foil from light penetration, until use at -18 degrees Celsius [17].

2-2-2-2 extraction with the help of ultrasound waves

The slag samples were affected by the ultrasonic waves of the sonication process with a frequency of 25 kHz at times of 10, 25 and 40 minutes, and sound intensity of 30, 60 and 90% [18]. In the next step, the samples treated by enzymatic method were simultaneously affected by ultrasound waves to investigate the effect of enzymatic treatment, ultrasound waves and the combined effect of enzymatic treatment and ultrasound waves on the content of extracted phenolic compounds.

2-2-3 Tests performed on the extract

2-2-3-1 Determination of extraction yield

The extraction efficiency was based on the content of total phenol in 100 grams of sorghum pulp. After removing the solvent from the ethanol extract, it was mixed with water and filtered through Whatman paper (No. 1) to remove its suspended particles. Then the amount of phenolic compounds according to the method(Folin–Ciocalteu) It was measured. The absorbance was read with a spectrophotometer at a wavelength of 765 nm and a calibration curve was drawn using gallic acid as a standard. The total phenol content in the sample was reported based on gallic acid equivalent [19].

2-2-3-2 determination of antioxidant activity by different methods

Inhibition of free radicals DPPH

The antiradical property of the extracted extract based on the ability to donate hydrogen atom or electron in ethanol extracts or the degree of decolorization of 2-diphenyl-1-picrylhydrazyl purple solution. (DPPH) It was measured in methanol [20].

Method FRAP

This test was performed based on the ability to regenerate trivalent iron ion and convert it into divalent iron ion. Divalent iron obtained inpH acid and in the presence of a reagentTPTZ، formation Fe-TPTZ The data has a blue color and the intensity of the resulting color can be measured at a wavelength of 593 nm with a spectrophotometer. For this purpose, the concentration of 250 micrograms per milliliter was taken from the sample to the final volumeml 2 solutions TPTZ which contains ml 10 solutionsFRAPAt(mM HCl 40) Iron chloridemM 20 and BafrastatmM 300 with 3/6pH = was, added and kept at room temperature for 10 minutes ^{O}C 37 is placed and the intensity of the resulting color in the wavelengthnm 593 was read against the blank. To draw a standard curve of ferrous sulfate with concentrations of 1000, 500, 250, 125 micromolar and antioxidant power based on.

Micromole scale $Fe^{+2}$was stated From the antioxidant compound quercetin[1] was used as a positive control [21].

MethodTEAC

for the preparation of the radical 2 and 2 - azino base - 3- Ethyl benzothiazoline 6- Sulphonic acid[2] (ABTS)، First, an aqueous solution with a concentration of 7 mmol was prepared. to this solution ABTS، Potassium persulfate was added until its final concentration reached 2.45 mmol in the solution. The resulting solution was kept at room temperature and darkness for 16 hours. In this period of the moleculeABTS Radical cation ABTS was produced Take 20 microliters of the samples with a pipette and add 2 milliliters of the solution $ABTS^{\bullet+}$ Derkot was mixed, then its absorption at 734 nm was read at 2, 4 and 6 minutes after mixing. Results in numbersTEAC Radical scavenging powerABTS Samples based on standard Trolox It was stated [22].

2-2-3-3 Measurement of total acidity andpH

Total acidity by titration and measurementpH by usingpH m for the extracted extracts [23].

2-2-3-4 determination of the amount of total flavonoids

Aluminum chloride colorimetric method was used to determine the amount of flavonoids. to 0.5 ml of each extract (10 mg/ml), 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride solution in ethanol, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water was added. The absorbance of the mixture was read at 415 nm against a blank after 30 minutes of storage at room temperature. Quercetin was used as a standard to draw the calibration curve. The amount of flavonoid was reported based on the amount equivalent to milligrams of quercetin per gram of extract [24].

2-2-3-5 Identification of extracted compounds in the optimal sample

Quantitative and qualitative investigation and identification of phenolic compounds in the extracted extract of the optimal slag pulp using a high-performance liquid chromatography device.(HPLC) Done. column (μm 5 andmm 6/4 * mm 250) ODS C_{18}، For analytical separation and diode array detector(DAD) in systemHPLC (Agilent 1260 infinity) used. For the detection and quantification of phenolic compounds, standard concentrations of 2.5, 5, 10, 20 and 40 mg/liter for the calibration curves of gallic acid, vanillic acid, caffeic acid, caffeic acid, acid-pCoumaric, ferulic acid, sinapic acid, quercetin and epicatechin were used. Concentrations of 5, 10, 20, 40 and 60 mg/L were used for calibration curves of catechin, epigallocatechin and epigallocatechin gallate. Extracts were diluted with methanol before analysisHPLC through a syringe filterPTFE 45/0 They were micrometer filtered. Then samples directly to the systemHPLC were injected [25].

2-2-4 statistical analysis method

Experiment data with one-way analysis of variance (One-way ANOVA) were compared Statistically significant differences between mean values (in cases where the overall effect of treatments is significant) were determined using Duncan's multi-range follow-up test. Statistical tests of the results obtained using the softwareSPSS Version 26 was done. The significance level is $0.05 \geq p$ was considered for all data comparisons.

3- Results and discussion

3-1 The results of tests of polyphenol extract extracted under ultrasound and pectolytic enzymes

3-1-1 Extraction efficiency

[1]

[2] 2,2′--Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

The results of the present realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects on the extraction efficiency of polyphenolic extract was significant. ($p\leq0.01$). According to the invoiceF ‹The effect of extraction time (692/658) on changes in polyphenol compounds extraction efficiency was more significant than other factors.($p\leq0.05$). The highest extraction efficiency belonged to the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 40 minutes. ($p\leq0.01$). After that, the samples extracted by Yildamesh enzyme and under ultrasound at 60% sound intensity for 40 minutes and then the sample extracted by Ultracolor enzyme and under ultrasound at 60% sound intensity for 40 minutes had the highest extraction efficiency. ($p\leq0.01$). The sample without enzyme and subjected to ultrasound at 30% sound intensity for 10 minutes had the lowest extraction efficiency. ($p\leq0.01$). It has been reported in the articles that ultrasound intensity has a positive effect on the yield of polyphenols, which is attributed to the collision of molecules during the increase in ultrasound power, which facilitates the release of phenolic compounds. Ultrasonic waves create tiny air bubbles inside the liquid environment and then burst them, which is called cavitation. The asymmetric bursting of these bubbles in the vicinity of the food substance causes rapid and explosive currents of sound waves to be transmitted to its surface, and by causing successive contractions and expansions in it, and also based on the theories in the extraction processes, due to the explosion of the bubbles in the sonication process. In the aqueous solution, the destruction of the cell wall and the creation of microscopic narrow channels in the plant tissue have been done, which ultimately causes the water to exit the product more easily, and with it, the effective substances of the plant.]26[.Balasubramaniam et al. (2019), in the study of the effect of the time factor on the extraction efficiency of polyphenolic compounds, they stated that the extraction efficiency reached the maximum in 30 minutes and further decreased, and they acknowledged that long-term extraction at high temperatures can reduce the level of diffusion and the speed of diffusion. and lead to polymerization or destruction of heat-sensitive polyphenols, and as a result, it may reduce the yield. On the other hand, increasing the power from 250 to 500 watts led to an increase in efficiency]27[. Observations made byChavan AndSinghal (2013) indicated the increase in polyphenolic arcanate efficiency by increasing the ultrasonication time up to 35 minutes, after which a decrease in extraction efficiency was observed.]28[. TEA AndBrich(2014) concluded that extraction with the help of sonication for 20 minutes resulted in a high yield of phenols and flavonoid contents from rape seed cakes, after which there was a decrease in content with respect to time.]29[. But in the present research, by increasing the duration of sounding from 10 to 40 minutes, the extraction efficiency increased significantly. The results of this research are in accordance with the reportsLingzhuand colleagues (2015) who stated that during the extraction of corn cob polyphenols, with an increase

The ultrasonic power increased the extraction efficiency, which proves that the ultrasonic power and frequency play a dynamic role in the extraction of polyphenols.]30[. Martino et al. (2006) in the study of the effect of microwave, ultrasound and soxhlet in the extraction of coumarin and similar compounds from clover plant stated that during the extraction with ultrasound, the best extraction mode was obtained as a result of the time of 60 minutes and the use of ethanol solvent, which compared to the method Soxhlet had more extraction efficiency (quantitative and qualitative) [31]. Jimenez et al. (2007) in the study of the effect of oil extraction from milled olive seeds by high power ultrasonic waves, stated that in the presence of these waves, the walls of plant cells and tissues are destroyed and antioxidant compounds (tocopherols, polyphenols) and pigments (more chlorophyll and carotenoid) entered the oil, which led to an increase in the nutritional value of the desired oil [32]. According to the findings of the researchers, the dual function of hydrolysis by enzymes along with ultrasound results in the extraction of extracts with more phenolic content compared to pre-treatment with the enzyme, which is attributed to the release of more bound and unbound phenols [33].According to the findings of the researchers, increasing the effect of cavitation by increasing the intensity of ultrasound in fruit peel extract*Nephilim lapasium*[3] And also in grape seeds when the intensity of the sound fromIN50 to IN150 increased, which ultimately led to an increase in total phenol content tour]34 ،35[. Different intensities of ultrasound were used in the extraction of polyphenols from crushed tea leaves and by increasing the power from 25 to 125 watts, the efficiency increased by 16.6%.]36[. In addition to increasing the extraction of polyphenols, it was observed that hydroxyl radicals (OH*) which are produced at high sound intensity, especially in the presence of high water content, they can react with phenols and lead to their decomposition, so caution should be taken to prevent the destruction of polyphenols by using high sound intensity]37 ،38[.

[3]-*Nephelium lappaceum*

Table 1. Analysis of variance of the effect of treatments on Extraction efficiency of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	111.006	55.503	2.637	0.000 **
UltraSound intensity	2	17.432	8.716	414.064	0.000 **
extraction time	2	29.160	14.580	692.658	0.000 **
Enzyme x sound intensity	4	0.406	0.102	4.826	0.002 **
Enzyme × time	4	0.838	0.210	9.955	0.000 **
UltraSound intensity x time	4	0.642	0.161	7.628	0.000 **
Enzyme × sound intensity × time	8	0.734	0.092	4.358	0.000 **
error	54	1.137	0.021		
Total	81	8170.412			

**Significance at 1% probability level, *Significance at 5% probability level

3-3-2 Evaluation of pH results

The results of this realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects on pH It was meaningful($p\leq0.01$). According to the invoiceF, the effect of sound intensity (146/514) on changespH It was more significant than other factors ($p\leq0.01$) and the interaction effect of enzyme × time (2.959) has the lowest effect on the amountpH had($p\leq0.05$). The lowest amount pHIt belonged to the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 40 minutes and the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 25 minutes.($p\leq0.01$). The sample without enzyme and under ultrasound at 30% sound intensity for 10 minutes has the highest amountpH Was($p\leq0.01$). According to the results, the lowestpH It belonged to Yaldemesh enzyme($p\leq0.01$) and with increasing sound intensity,pH It decreased significantly($p\leq0.01$). On the other hand, increasing the extraction time leads to a significant decreasepH became ($p\leq0.01$). which may be attributed to the extraction efficiency and the total phenol content of the desired extracts over time, which leads to a decreasepH The samples have been

Table 2. Analysis of variance of the effect of treatments on pH of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	0.582	0.291	6.366 E3	0.000 **
UltraSound intensity	2	0.013	0.007	146.514	0.000 **
extraction time	2	0.145	0.073	1.590 E3	0.000 **
Enzyme x sound intensity	4	0.002	0.001	12.324	0.000 **
Enzyme × time	4	0.001	0.000	2.959	0.028 **
UltraSound intensity x time	4	0.009	0.002	49.662	0.000 **
Enzyme × sound intensity × time	8	0.002	0.000	4.986	0.000 **
Error	54	0.002	4,568 E-5		
Total	81	903.1504			

**Significance at 1% probability level, *Significance at 5% probability level

3-3-3 Evaluation of acidity results

The results of the present realization showed that the effect of enzyme type, sound intensity, extraction time and the interaction effect of enzyme x sound intensity on acidity was significant.($p\leq0.01$). According to the invoiceFEnzyme type (505/368) was more significant on acidity changes than other factors.($p\leq0.01$) And the

interaction effects of enzyme × time, sound intensity × time, enzyme × sound intensity × time were not significant on acidity level.($p\geq0.05$). The highest amount of acidity belonged to the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 40 minutes and the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 25 minutes.($p\leq0.01$). The sample without enzyme and subjected to ultrasound at 30% sound intensity for 10 minutes had the lowest level of acidity.($p\leq0.01$). According to the obtained results, the highest acidity belonged to the enzyme Yaldemesh($p\leq0.01$) And with increasing sound intensity, acidity increased significantly($p\leq0.01$). On the other hand, increasing the extraction time led to a significant increase in acidity($p\leq0.01$).

Table 3. Comparison of Extraction efficiency, pH and acidity mean of sour grape waste extracts

Enzyme type	UltraSound intensity (%)	Time (min)	Extraction efficiency (%)	pH	Acidity (%)
	30	10	[g] 0.08 ± 7.26	[a] 0.01 ± 4.51	[k] 0.008± 0.426
		25	[pq] 0.32 ± 7.57	[b] 0.01 ± 4.44	[jk] 0.008± 0.431
Without Enzyme		40	[O] 0. 12 ± 8.05	[c] 0.01 ± 4.39	[ij] 0.007± 0.444
	60	10	[on] 0.06 ± 7.89	[a] 0.01 ±4.49	[k] 0.008± 0.426
		25	[n] 0.07 ± 8.47	[c] 0.01 ± 4.39	[k] 0.008± 0.426
		40	[k] 0.08 ± 9.24	[c] 0.00 ± 4.38	[jk] 0.008± 0.439
	90	10	[O] 0.10 ± 8.03	[b] 0.01 ± 4.44	[k] 0.008± 0.426
		25	[m] 0.01 ± 8.65	[c] 0.01 ± 4.38	[jk] 0.007± 0.431
		40	[j] 0.12 ± 9.26	[c] 0.00 ± 4.38	[jk] 0.008± 0.439
Pectinex Ultra ColorEnzyme	30	10	[l] 0.02 ± 9.04	[d] 0.00 ± 4.36	[hi] 0.007± 0.457
		25	[i] 0.03 ± 9.95	[f] 0.00 ± 4.29	[gh] 0.008± 0.465
		40	[fg] 0.34 ± 10.52	[hi] 0.01 ± 4.24	[cd] 0.007± 0.495
	60	10	[j] 0.08 ± 9.68	[d] 0.00 ± 4.36	[defgh] 0.013± 0.474
		25	[f] 0.05 ± 10.69	[g] 0.01 ± 4.27	[efg] 0.007± 0.478
		40	[b] 0.02 ± 11.86	[i] 0.01 ± 4.22	[of] 0.008± 0.490
	90	10	[gh] 0.09 ± 10.23	[It is] 0.00 ± 4.33	[def] 0.007± 0.482
		25	[of] 0.18 ± 11.19	[gh] 0.01 ± 4.25	[cd] 0.007± 0.495
		40	[bc] 0.17 ± 11.69	[gh] 0.01 ± 4.25	[bc] 0.008± 0.507
Pectinex YildMASH.	30	10	[j] 0.17 ± 9.69	[f] 0.01 ± 4.30	[fgh] 0.008± 0.469
		25	[f] 0.19 ± 10.66	[i] 0.01 ± 4.22	[of] 0.010± 0.488
		40	[of] 0.10 ± 11.08	[k] 0.01 ± 4.17	[of] 0.008± 0.490
	60	10	[hi] 0.09 ± 10.07	[f] 0.00 ± 4.29	[cd] 0.007± 0.495
		25	[d] 0.06 ± 11.24	[j] 0.00 ± 4.19	[cd] 0.010± 0.497
		40	[b] 0.08 ± 11.76	[j] 0.00 ± 4.19	[abc] 0.015± 0.516
		10	[if] 0.30 ± 10.81	[hi] 0.01 ± 4.24	[bc] 0.007± 0.508
	90	25	[c] 0.10 ± 11.48	[j] 0.01 ± 4.19	[ab] 0.007± 0.521
		40	[a] 0.14 ± 12.08	[k] 0.01 ± 4.17	[b] 0.008± 0.529

*Results are reported as mean ± standard deviation. The presence of at least one similar Latin letter in each column indicates that there is no significant difference between the values at the 95% confidence level.

Table 4. Analysis of variance of the effect of treatments on acidity of sour grape waste extracts

Source of changes	DF	sum of the	mean of the	F	Sign

		squares	squares		
Enzyme type	2	0.070	0.035	505.368	0.000**
UltraSound intensity	2	0.005	0.002	36.265	0.000**
extraction time	2	0.006	0.003	43.699	0.000**
Enzyme x sound intensity	4	0.003	0.001	12.270	0.000**
Enzyme × time	4	0.000	0.000	1.652	0.175ns
UltraSound intensity x time	4	0.000	6.969E-5	1.013	0.409ns
Enzyme × sound intensity × time	8	0.000	6.244E-5	0.907	0.517ns
error	54	0.004	6.881E-5		
Total	81	18.140			

**Significance at 1% probability level, ns: Not significance

3-3-4 Evaluating the results of antioxidant activity with different methods

3-3-4-1 inhibition of free radicalsDPPH

The results of the present realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects on antioxidant activity (by free radical inhibition methodDPPH) was significant($p\leq0.01$). According to the invoiceF, the interaction effect of enzyme × time (79/282) on changes in antioxidant activity (by free radical inhibition methodDPPH) was more significant than other factors($p\leq0.01$). The highest level of antioxidant activity (by inhibiting free radicalsDPPH) belonged to the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 40 minutes and the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 25 minutes.($p\leq0.01$). The sample without enzyme and subjected to ultrasound at a sound intensity of 30% for 10 minutes has the lowest antioxidant activity (by free radical inhibition methodDPPH) Was($p\leq0.01$). According to the obtained results, the highest antioxidant activity (by inhibiting free radicalsDPPH) belonged to Yaldemesh enzyme and by increasing the sound intensity, the antioxidant activity (by inhibiting free radicals)DPPH) increased significantly($p\leq0.01$). On the other hand, increasing the extraction time leads to a significant increase in antioxidant activity (by inhibiting free radicalsDPPH) became($p\leq0.01$). The reason for this can be attributed to the amount of phenolic compounds. Polyphenols are known for their ability to perform a series of oxidation and reduction reactions and for local resonance effects in phenyl rings. Polyphenols are considered more efficient antioxidants due to their diverse chemical structures, from simple to complex [39]. Several studies have been conducted in the field of evaluating the antioxidant activity of plant polyphenols extracted by ultrasound treatment [40, 41, 42, 43]. Measurement valueTEAC Flower extract*Limonium sinuatum* obtained by using ultrasound was higher than soaking and Soxhlet method [44]. Other studies also showed higher antioxidant activity of extracts extracted by ultrasound from different plant sources compared to conventional methods. According to reportsTEA AndBirch (2014), phenols and flavonoids extracted with the help of ultrasonic waves from fat-free flax cakes and rapeseeds had twice the antioxidant potential than conventional extraction methods, these results show the preference of ultrasound treatment in the extraction of polyphenols from plants compared to Traditional methods correspond [29].

Table 5. Analysis of variance of the effect of treatments on antioxidant capacity (DPPH) of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	1466.207	733.104	3.038 E3	0.000 **
UltraSound intensity	2	619.446	309.723	1.284 E3	0.000 **
Extraction time	2	1683.741	841.871	3.489 E3	0.000 **
Enzyme × ultrasound intensity	4	61.950	15.488	64.181	0.000 **
Enzyme × time	4	76.525	19.131	79.282	0.000 **
UltraSound intensity × time	4	37.346	9.336	38.691	0.000 **
Enzyme × ultrasound intensity × time	8	39.955	4.994	20.697	0.000 **
Error	54	13.031	0.241		
Total	81	93447.047			

**Significance at 1% probability level

3-3-4-2 Iron recovery rate (FRAP method)

The results of the present realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects on antioxidant activity (iron recovery rate, methodFRAP) was significant($p\leq0.01$). According to the invoiceF, the interaction effect of enzyme × sound intensity × time (114/676) on the changes of antioxidant activity (iron recovery rate, methodFRAP) was more significant than other factors($p\leq0.01$). The highest amount of antioxidant activity (iron recovery rate, methodFRAP) belonged to the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 40 minutes and the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 25 minutes.($p\leq0.01$). The sample without enzyme and subjected to ultrasound at 30% sound intensity for 10 minutes has the lowest antioxidant activity (iron recovery rate, methodFRAP) Was($p\leq0.01$). According to the obtained results, the highest antioxidant activity (iron recovery rate, methodFRAP) belonged to the Yldmesh enzyme($p\leq0.01$) And with increasing sound intensity, antioxidant activity (iron regeneration rate, methodFRAP) increased significantly($p\leq0.01$) On the other hand, increasing the extraction time leads to a significant increase in antioxidant activity (iron recovery rate, methodFRAP) became($p\leq0.01$).

Table 6. Analysis of variance of the effect of treatments on antioxidant capacity (FRAP) of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	0.001	0.000	1.723 E-4	0.000 **
UltraSound intensity	2	0.000	0.000	6.582 E-3	0.000 **
Extraction time	2	0.001	0.001	2.451 E-4	0.000 **
Enzyme × ultrasound intensity	4	1,720 E-5	4.301 E-6	175.057	0.000 **
Enzyme × time	4	7,867 E-5	1.967 E-5	800.553	0.000 **
UltraSound intensity × time	4	6,858 E-6	1.715 E-6	69.789	0.000 **
Enzyme × ultrasound intensity × time	8	2,254 E-5	2.817 E-6	114.676	0.000 **
Error	54	1.327	2.457 E-8		
Total	81	0.086			

**Significance at 1% probability level

3-3-4-3 ABTS radical absorption method

The results of the present realization showed that the effect of enzyme type,

sound intensity, extraction time, and their mutual effects on antioxidant activity (radical absorption methodABTS) was significant($p\leq0.01$). According to factor F, the effect of enzyme type (937/019) on changes in antioxidant activity (radical absorption methodABTS) was more significant than other factors($p\leq0.05$). The highest level of antioxidant activity (radical absorption methodABTS(belonged to the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 40 minutes and the sample extracted by Yıldımş enzyme and under ultrasound at 90% sound intensity for 25 minutes)$p\leq0.01$). The sample without enzyme and subjected to ultrasound at 30% sound intensity for 10 minutes has the lowest antioxidant activity (radical absorption methodABTS) Was($p\leq0.01$). According to the obtained results, the highest antioxidant activity (radical absorption methodABTS) belonged to the Yldmesh enzyme($p\leq0.01$) And with increasing sound intensity, antioxidant activity (radical absorption methodABTS) increased significantly($p\leq0.01$) On the other hand, increasing the extraction time leads to a significant increase in antioxidant activity (radical absorption methodABTS) became($p\leq0.01$). Excuse meand colleagues (2012) in investigating the effect of extraction conditions with the help of ultrasound on the quality of the extracts obtained from Mesembrintum plant.[4] They stated that for the two solvents used, longer extraction time resulted in higher polyphenol content, so that longer ultrasound duration (10 minutes) resulted in extracts with higher antioxidant activity and in general they stated which antioxidant capacity*M. to success* It is strongly influenced by the nature of the extracting solvent and the duration of ultrasonic extraction [45].

[4]- Mesembryanthemum success

Table 7. Analysis of variance of the effect of treatments on antioxidant capacity (ABTS) of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	1708.859	854.430	937.019	0.000 **
UltraSound intensity	2	1527.021	736.511	837.312	0.000 **
Extraction time	2	3567.585	1783.793	1.956 E3	0.000 **
Enzyme × ultrasound intensity	4	134.001	33.500	36.738	0.000 **
Enzyme × time	4	348.571	87.143	95.566	0.000 **
UltraSound intensity × time	4	315.708	78.927	86.556	0.000 **
Enzyme × ultrasound intensity × time	8	218.851	27.356	30.001	0.000 **
Error	54	49.240	0.912		
Total	81	120313.810			

**Significance at 1% probability level

Table 8. Comparison of antioxidant capacity mean of sour grape waste extracts

Enzyme type	UltraSound intensity (%)	Time (min)	DPPH (mg/mL)	FRAP (mg/g)	ABTS (mg/mL)
	30	10	[a] 0.01±53.27	[p] 0.0002±0.0184	[a] 1.59±69.77
		25	[c] 0.35±41.14	[m] 0.0001±0.0284	[c] 1.68±44.06
Without Enzyme		40	[d] 0.49±36.94	[j] 0.0001±0.0310	[efg] 1.22±37.58
	60	10	[b] 1.45±44.24	[O] 0.0001±0.0208	[b] 1.90±54.84
		25	[of] 0.27± 36.29	[k] 0.0002±0.0300	[of] 0.47±38.64
		40	[gh] 0.58± 3234	[fg] 0.0002±0.0333	[h] 0.38±33.99
	90	10	[c] 0.60±40.74	[n] 0.0001±0.0239	[c] 1.59±43.00
		25	[g] 0.46±33.25	[h] 0.0001±0.0316	[g] 0.42±36.05
		40	[j] 0.33±30.11	[d] 0.0001±0.0352	[j] 0.31±31.04
Pectinex Ultra ColorEnzyme	30	10	[c] 1.07±41.38	[n] 0.0002±0.0242	[b] 2.51±52.88
		25	[f] 0.41±34.44	[i] 0.0001±0.0313	[if] 0.94±37.79
		40	[k] 0.28±28.74	[It is] 0.0001±0.0343	[h] 0.38±33.39
	60	10	[c] 0.78±40.86	[m] 0.0002±0.0283	[c] 1.13±44.25
		25	[h] 0.32±32.30	[g] 0.0003±0.0329	[g] 0.75±35.95
		40	[l] 0.13±27.16	[c] 0.0002±0.0368	[j] 0.32±30.99
	90	10	[of] 0.46±36.08	[k] 0.0001±0.0299	[d] 0.53±39.51
		25	[m] 0.12±26.64	[c] 0.0001±0.0371	[l] 0.25±27.09
		40	[m] 0.07±26.40	[c] 0.0001±0.0371	[l] 0.24±26.81
Pectinex YildMASH.	30	10	[It is] 0.26±35.98	[l] 0.0002±0.0293	[c] 0.65±43.25
		25	[i] 0.20±31.53	[g] 0.0001±0.0332	[ij] 0.35±31.68
		40	[l] 0.14±27.18	[d] 0.0003±0.0355	[k] 0.31±29.78
	60	10	[h] 0.36±32.11	[f] 0.0002±0.0336	[fg] 0.46±36.56
		25	[m] 0.50±26.20	[b] 0.0002±0.0390	[h] 0.39±33.38
		40	[n] 0.25±25.43	[b] 0.0002±0.0394	[i] 0.36±32.29
		10	[j] 0.18±30.21	[g] 0.0001±0.0332	[h] 0.40±34.06
	90	25	[O] 0.11±23.25	[a] 0.0003±0.0402	[m] 0.21±23.79
		40	23.05±0.10 [O]	0.0404± 0.0001 [a]	23.57±0.19 [m]

*Results are reported as mean ± standard deviation. The presence of at least one similar Latin letter in each column indicates that there is no significant difference between the values at the 95% confidence level.

3-3-4-4 Evaluation of total flavonoid results

The results of this realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects were on total flavonoids.($p \leq 0.01$). According to the invoiceF, the interaction effect of enzyme × time (47.022) on total flavonoid changes) was more significant than other factors.($p \leq 0.01$). The highest amount of total flavonoid belonged to the sample extracted by Yildamesh enzyme and subjected to ultrasound at 90% sound intensity for 40 minutes, and then the sample extracted by Yildamesh enzyme and subjected to ultrasound at 90% sound intensity for 25 minutes had the highest amount. It was rich in flavonoids($p \leq 0.01$). The sample without enzyme and subjected to ultrasound at 30% sound intensity for 10 minutes had the lowest total flavonoids.($p \leq 0.01$). According to the obtained results, the highest total flavonoid belonged to Yaldemesh enzyme($p \leq 0.01$) And with increasing sound intensity, total flavonoids increased significantly($p \leq 0.01$) On the other hand, increasing the extraction time led to a significant increase in total flavonoids($p \leq 0.01$). Flavonoids are a group of polyphenol compounds that are produced in plants as secondary metabolites. Flavonoids are widely found in fruits, vegetables and other food products. They have favorable biochemical effects on many diseases (such as cardiovascular diseases, arteriosclerosis) and also other biological activities (such as anti-inflammatory, anti-aging) [46, 47]. The main biological activity of flavonoids, which has been widely studied, is their antioxidant activity. The antioxidant activity of flavonoids can prevent the damage caused by free radicals by inhibiting reactive oxygen species (ROS), activation of antioxidant enzymes, inhibition of oxidases (such as xanthine oxidase [XO],Cyclooxygenase [COX] ، Lipoxygenazit[PI3Kinophos]) It prevents and leads to the reduction of radicalsa-be tocopheryl Antioxidant activity of flavonoids can increase uric acid levels, metal chelating activity and low molecular weight antioxidant activity to reduce oxidative stress [48].Wang et al. (2023) in investigating the effect of ultrasound along with γ-aminobutyric acid treatmentFRONT Exogenous effect on polyphenolic metabolites and antioxidant activity of mung beans during germination, mung beans with a combination of ultrasound andFRONT stated that the combined treatments significantly increased the content of polyphenols and free flavonoids of mung bean sprouts depending on the duration of germination. In addition, a positive correlation between polyphenol content and antioxidant activityin vitro Mung sprouts were found [49].

Table 9. Analysis of variance of the effect of treatments on antioxidant capacity(ABTS) of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	1708.859	854.430	937.019	0.000 **
UltraSound intensity	2	1527.021	736.511	837.312	0.000 **
Extraction time	2	3567.585	1783.793	1.956 E3	0.000 **
Enzyme × ultrasound intensity	4	134.001	33.500	36.738	0.000 **
Enzyme × time	4	348.571	87.143	95.566	0.000 **
UltraSound intensity × time	4	315.708	78.927	86.556	0.000 **
Enzyme × ultrasound intensity × time	8	218.851	27.356	30.001	0.000 **
Error	54	49.240	0.912		
Total	81	120313.810			

**Significance at 1% probability level

3-3-4-5 Evaluating the results of determining the amount of total phenol

The results of the present realization showed that the effect of enzyme type, sound intensity, extraction time, and their mutual effects were on total phenol($p\leq0.01$). According to the invoiceF, the effect of extraction time (951/016) on total phenol changes was more significant than other factors.($p\leq0.01$). The highest amount of total phenol belonged to the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 40 minutes, and then the sample extracted by Yildamesh enzyme and under ultrasound at 90% sound intensity for 25 minutes had the highest amount. It was total phenol($p\leq0.01$). The sample without enzyme and under ultrasound at 30% sound intensity for 10 minutes had the lowest total phenol.($p\leq0.01$). According to the obtained results, the highest total phenol belonged to Yaldemesh enzyme($p\leq0.01$) And with increasing sound intensity, total phenol increased significantly($p\leq0.01$) On the other hand, increasing the extraction time led to a significant increase in total phenol($p\leq0.01$). The results of various researches have shown that different enzymes are suitable for use in different phenolic matrices and the use of these enzymes has only improved phenolic compounds. [50, 51]. Among the reasons for the low extraction of phenolic compounds from grapes to wine, even when some enzymes are used, the interaction between the extracted phenolic compounds and the cell walls of the skin and suspended pulp has been suggested. Cell wall components show a high tendency to phenolic compounds and absorb them in their structure [52, 53, 54]. Structural polysaccharides that have the highest affinity for combining with phenolic compounds include pectin, hemicellulose, and partially cellulose. [55]. Therefore, by crushing grapes, phenolic compounds are extracted, but a large amount of suspended cell wall material is also produced. StudiesOsete-Alcaraz et al. (2020)Bondet al., (2010) showed that phenolic compounds bind to suspended cellular material. When this binding occurs, these interactions may reduce the phenolic concentration in the final wine.]57 ،56[. Osete-Alcaraz et al. (2021) showed that when the purified grape skin cell wall comes into contact with pure pectolytic or commercial pectolytic enzymes, a significant degree of cell wall depectinization opens the cell walls for the release of phenolic compounds [58]. Similar results in grape samples byZietsman et al. (2015) found that changes occurred in the skin of grape seeds during soaking and fermentation in the presence of enzymes, and the researchers observed that in the presence of enzymes, the depectinization of

cell walls increased and they opened up.]59[. Luet al. (2017) found that mixed/complex enzymes improved phenolic extraction from guava leaves compared to single cellulase enzyme [60]. Also, Mushtaq et al., (2015) stated that supercritical extraction with the help of enzymes (cellulase, pectinase and protease) led to a twofold increase of polyphenols extracted from pomegranate peel [61].Balasubramaniam et al. (2019), stated that the total polyphenol content increased dramatically with increasing enzyme concentration up to 1500 U, and then the phenolic levels decreased, and the reason for this was attributed to substrate limitation at higher enzyme concentrations. The purpose of enzyme pretreatment is to disrupt the plant cell wall structure by weakening/decomposing the cellulose-phenolic network in order to enable the release and recovery of phenolic bioactive substances [27].Shahramet al. (2018), in investigating the effect of pectinase concentration, sonication time, etcpH On the extraction of phenolic compounds from orange processing wastes, they stated that at 0% concentration of pectinase,TPC Extracts increased with increasing sonication time from 10 to 120 minutes. The increase in the amount of phenolic compounds extracted by increasing the ultrasonic time was attributed to the effect of ultrasonic waves on breaking the cell wall of the sample and as a result, increasing the interface between the solvent and the cell wall [62]. In addition, by increasing the ultrasonication time, the interaction of phenolic compounds with protein and polysaccharide compounds was weakened. This phenomenon led to the delivery of more phenolic compounds from the plant sample to the solvent [63]. With increasing pectinase concentration, the amount of phenolic compounds decreased. Probably, the use of high concentrations of pectinase enzymes has a negative effect on the extraction of the total phenolic compounds of the extracts and destroys or reduces the final phenolic compounds [64].Khan et al. (2010) in the study of extraction of polyphenolic compounds of orange peel using ultrasound process stated that by increasing the extraction time from 10 to 60 minutes,TPC gradually increased [65]. According to the reports of researchers, ultrasound not only leads to an increase in the extraction efficiency of polyphenols, but also preserves and increases the biological activity of polyphenol extracts compared to traditional soaking and Soxhlet extraction methods [66].

Table 10. Analysis of variance of the effect of treatments on antioxidant capacity (ABTS) of sour grape waste extracts

Source of changes	DF	sum of the squares	mean of the squares	F	Sign
Enzyme type	2	1708.859	854.430	937.019	0.000 **
UltraSound intensity	2	1527.021	736.511	837.312	0.000 **
Extraction time	2	3567.585	1783.793	1.956 E3	0.000 **
Enzyme × ultrasound intensity	4	134.001	33.500	36.738	0.000 **
Enzyme × time	4	348.571	87.143	95.566	0.000 **
UltraSound intensity × time	4	315.708	78.927	86.556	0.000 **
Enzyme × ultrasound intensity × time	8	218.851	27.356	30.001	0.000 **
Error	54	49.240	0.912		
Total	81	120313.810			

**Significance at 1% probability leve

Table 11. Comparison of total phenol&flavonoid mean of sour grape waste extracts

Enzyme type	UltraSound intensity (%)	Time (min)	Total flavonoid (mg/mL)	Total phenol (mg/mL)

	30	10	[in] 0.02±2.44	[in] 0.02±5.11
		25	[t] 0.02±2.55	[t] 0.03±5.23
		40	[r] 0.02±2.70	[r] 0.03±5,44
Without Enzyme	60	10	[s] 0.01±2.66	[s] 0.02±5.32
		25	[q] 0.01±2.75	[q] 0.03±5.51
		40	[p] 0.02±2.88	[O] 0.03±5.73
	90	10	[p] 0.02±2,86	[p] 0.04±5.60
		25	[O] 0.02±3.00	[n] 0.02±5.97
		40	[n] 0.04±3.12	[m] 0.04±6.18
Pectinex Ultra Color Enzyme	30	10	[m] 0.03±3.77	[l] 0.02±7.83
		25	[l] 0.01±3.96	[k] 0.04±8.09
		40	[j] 0.01±4.20	[j] 0.03±8.38
	60	10	[l] 0.03±3.92	[k] 0.02±8.14
		25	[jk] 0.04±4.17	[j] 0.03±8.35
		40	[i] 0.01±4.25	[hi] 0.18±8.64
	90	10	[h] 0.04±4.37	[i] 0.06±8.56
		25	[g] 0.02±4.50	[g] 0.05±8.98
		40	[fg] 0.02±4.54	[f] 0.05±9.10
Pectinex YildMASH.	30	10	[k] 0.01±4.16	[j] 0.02±8.30
		25	[h] 0.02±4.35	[i] 0.02±8.63
		40	[if] 0.04±4.60	[f] 0.03±9.12
	60	10	[It is] 0.03±4.62	[h] 0.03±8.83
		25	[c] 0.01±4.83	[d] 0.06±9.32
		40	[c] 0.06±4.83	[c] 0.04±9.50
	90	10	[d] 0.02±4.72	[It is] 0.04±9.21
		25	[b] 0.02±5.17	[b] 0.05±9.88
		40	[a] 0.01±5.21	[a] 0.04±9.99

*Results are reported as mean ± standard deviation. The presence of at least one similar Latin letter in each column indicates that there is no significant difference between the values at the 95% confidence level.

3-3-4-6 The results of the analysis of phenolic compounds in the extract of Ghor wastesby deviceHPLC

Extracted extracts were injected into a high performance liquid chromatography device to identify phenolic compounds and the amount and type of phenolic compounds were determined. The phenolic compounds of the extracts along with the abundance percentage of each component are presented in Table 12. The results of the investigationThe phenolic compounds of the extracts showed thatA number of 12 phenolic compounds were identified in the extract of Ghor wastes, which were gallic acid[5] (1.23%), caftaric acid[6](46.65%), Catechin[7] (4.85%), epigallocatechin[8] (6.34%), vanillic acid[9] (2.47%), caffeic[10] (1.08%), epigallocatechin gallate [11] (1.27%), epicatechin[12] (79/1 %), acidP–Kumarik[13] (0.15%), ferulic acid[14] (27/0 %), acidP–synapic[15] (24/0 %), quercetin[16] (35/0 %). Collar et al. (2018) in a review The changes of phenolic compounds and antioxidant activity of gour water during the production of concentrate, byHPLC, polyphenol compounds of gallic acid, catechin, epigallocatechin, vanillic acid, epigallocatechin gallate, epicatechin, caftaric acid, caffeic acid, acidp-Coumaric, ferulic acid, sinapic and quercetin were reported in it [67].

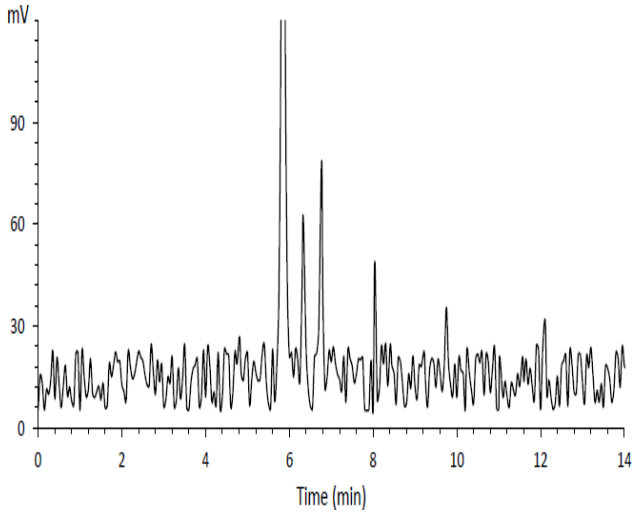

Chart 1. HPLC spectrum of phenolic compounds of of sour grape waste extracts

Table 12. The results of the type and amount of phenolic compounds of sour grape waste extracts

Type of compound (µg/g.dw)	Quantity (%)	Rt (min)
Gallic acid	1.23	4,81
Caftaric acid	46.65	5.85
Catechin	4.85	6.33
Epigalocathechin	6.34	6.77
Vanillic acid	2.47	8.04
Caffeic acid	1.08	8.41
Epigalocathechin gallate	1.27	9.22
Epicathechin	1.79	9.75
p-Cumaric acid	0.15	10.24
Ferolic acid	0.27	11.02
p-Cinapic acid	0.24	11.57
Querictin	0.35	12.11

[5]- Gallic

[6]- Caftaric acid

[7]- Catechin

[8]- Epigalocathechin

[9]- Vanillic acid

[10]- Caffeic acid

[11]- Epigalocathechin gallate

[12]- Epicathechin

[13]- p-Cumaric acid

[14]- Ferolic acid

[15]- Cinapic acid

[16]- Querictin

4-conclusion

The results of the present research showed that the highest extraction efficiency belonged to Yaldemesh enzyme and with increasing sound intensity, the extraction efficiency increased significantly. On the other hand, increasing the extraction time led to an increase in extraction efficiency. So that the highest extraction efficiency was observed in the sample extracted by Yaldemesh enzyme and under ultrasound at 90% sound intensity for 40 minutes. According to the obtained results, the highest flavonoid, total phenol and antioxidant activity belonged to Yaldemesh enzyme, and with increasing sound intensity, antioxidant activity increased significantly. On the other hand, increasing the extraction time led to a significant increase in antioxidant activity. In general, it can be said that by using pectinase enzyme along with ultrasound at the right time and sound intensity, the amount of total phenol and the antioxidant activity of polyphenolic extracts can be increased.

5- Appreciation and thanks

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تاثیر بکارگیری پیش تیمار فراصوت و آنزیم پکتیناز بر بازده استخراج و خواص آنتی اکسیدانی عصاره پلی فنلی ضایعات غوره (*Vitis viniferia*)

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چکیده

امروزه استخراج و بکارگیری ترکیبات فعال زیستی از پسماندهای کشاورزی و غذایی بسیار مورد توجه قرار گرفته است. در تحقیق حاضر اثر تیمار آنزیمی، فراصوت و اثر توام تیمار آنزیمی و امواج فراصوت بر استخراج ترکیبات فنلی ضایعات غوره انجام شد به طوری که از ۲ آنزیم پکتیناز (پکتینکس اولتراکالر و پکتینکس یلدمش) در سطوح۱۰، ۲۰ و ۳۰ میلی گرم بر کیلوگرم و فراصوت (زمان‌های ۱۰، ۲۵ و ۴۰ دقیقه) و (شدت صوت ۳۰، ۶۰ و ۹۰ درصد) استفاده شد. اثر تیمار آنزیمی، فراصوت و اثر توام تیمار آنزیمی و فراصوت بررسی شد. طبق نتایج به دست آمده، بالاترین بازده استخراج متعلق به آنزیم یلدمش بود و با افزایش شدت صوت، بازده استخراج به طور معنی‌داری افزایش یافت. از طرفی افزایش زمان استخراج، منجر به افزایش بازده استخراج شد ($p\leq0.01$). به طوری که بالاترین بازده استخراج در نمونه استخراج شده توسط آنزیم یلدمش و تحت فراصوت در شدت صوت ۹۰ درصد به مدت ۴۰ دقیقه مشاهده شد ($p\leq0.01$). طبق نتایج به دست آمده، بالاترین فلاونوئید، فنل تام و فعالیت آنتی‌اکسیدانی (DPPH، FRAP، ABTS) متعلق به آنزیم یلدمش بود و با افزایش شدت صوت، فعالیت آنتی اکسیدانی به طور معنی‌داری افزایش یافت ($p\leq0.01$). از طرفی افزایش زمان استخراج، منجر به افزایش معنی‌دار فعالیت آنتی‌اکسیدانی شد ($p\leq0.01$). بالاترین فعالیت آنتی‌اکسیدانی متعلق به نمونه استخراج شده توسط آنزیم یلدمش و تحت فراصوت در شدت صوت ۹۰ درصد به مدت ۴۰ دقیقه بود و به عنوان تیمار برتر معرفی شد.