DOI: 10.29050/harranziraat.1344099
Harran Tarım ve Gıda Bilimleri Derg. 2024, 28(3): 459-470

Research Article/Araştırma Makalesi

Evaluation of antioxidant activity and some physicochemical characteristics of pickled vine (*Vitis vinifera* L.) leaves

Salamura asma (Vitis vinifera L.) yapraklarının antioksidan aktivitelerinin ve bazı fizikokimyasal özelliklerin değerlendirilmesi

Tuba ZORLU ÜNLÜ[1], Semra TOPUZ[2*], Mustafa BAYRAM[3], Cemal KAYA[4]

[1] Department of Food Engineering, Instute of Graduate Studies, Tokat Gaziosmanpaşa University, 60150 Taşlıçiftlik, Tokat, Turkey
[2,3,4] Department of Food Engineering, Faculty of Engineering and Architecture, Tokat Gaziosmanpaşa University, 60150 Taşlıçiftlik, Tokat, Turkey

[1]https://orcid.org/0000-0001-8645-7228; [2]https://orcid.org/0000-0002-9122-0839; [3]https://orcid.org/0000-0002-8232-226X; [4]https://orcid.org/0000-0001-8354-9565

To cite this article:
Zorlu Ünlü, T., Topuz, S., Bayram, M. & Kaya, C. (2024). Evaluation of antioxidant activity and some physicochemical characteristics of pickled vine (*Vitis vinifera* L.) leaves. Harran Tarım ve Gıda Bilimleri Dergisi, 28(3): 459-470

DOI: 10.29050/harranziraat.1344099

***Address for Correspondence:**
Semra Topuz
e-mail:
semra.topuz@gop.edu.tr

Received Date:
17.08.2023
Accepted Date:
08.07.2024

ABSTRACT

Vine (*Vitis vinifera* L.) leaves have been used for centuries both to prepare various foods and for medicinal purposes. Vine leaves are processed to pickles for preserved for a long time. In this study, some physicochemical properties, total phenolic compounds and antioxidant activity of the pickled vine leaves produced by industrial or traditional methods from Narince variety grown in Tokat were determined. pH value (3.31-3.95), total acidity (0.3-1.72%), salt content (10.92-23.67%) were determined in pickled vine leaves produced by industrial or traditional methods. Total phenolic compounds for distilled water, 80% ethanol and 70% methanol extracts were determined in the range of 0.55-7.81 mg GAE g^{-1}, 3.98-15.66 mg GAE g^{-1}, 3.49-16.85 mg GAE g^{-1}, respectively. The cation radical scavenging activity (ABTS) for three solvents were determined in the range of 1.87-15.42 mg TE g^{-1}, 2.75-24.85 mg TE g^{-1}, 3.57-25.05 mg TE g^{-1}, while free radical scavenging activity (DPPH) for three solvents were determined in the range of 2.89-8.89 mg TE g^{-1}, 5.46-10.57 mg TE g^{-1}, 6.33-12.13 mg TE g^{-1}, respectively. As a result of the research, it was determined that the pickled vine leaves are rich source in terms of phenolic compounds and antioxidant activity but amount of salt in some samples was very high for consumption. The total phenolic compounds and antioxidant activity results of extracts obtained from pickled vine leaves using ethanol and methanol are close to each other but are higher than the results obtained distilled water.

Key Words: Antioxidant activity, Narince, Vine leaves, Phenolic compound, Physicochemical character

ÖZ

Asma (*Vitis vinifera* L.) yaprakları yüzyıllardır hem çeşitli yemeklerin hazırlanmasında hem de tıbbi amaçlarla kullanılmaktadır. Asma yaprakları uzun süre muhafaza edilmek amacıyla salamuraya işlenmektedir. Bu çalışmada, Tokat yöresinde yetiştirilen Narince çeşidinden endüstriyel ve geleneksel yöntemlerle üretilen salamura asma yapraklarının bazı fizikokimyasal özellikleri, toplam fenolik madde miktarları ve antioksidan aktiveleri belirlenmiştir. Endüstriyel ve geleneksel yöntemlerle üretilen salamura asma yapraklarının pH değerleri 3.31-3.95, toplam asitlik miktarı %0.3-1.72, tuz miktarı %10.92-23.67 aralığında tespit edilmiştir. Toplam fenolik madde miktarları saf su, %80 etil alkol ve %70 metil alkol ekstraktları için sırasıyla, 0.55-7.81 mg GAE g^{-1}, 5.00-15.66 mg GAE g^{-1}, 3.49-16.85 mg GAE g^{-1} aralığında belirlenmiştir. Katyon radikali giderme aktivitesi (ABTS) üç farklı çözücü için sırasıyla 1.87-15.42 mg TE g^{-1}, 2.75-24.85 mg TE g^{-1}, 3.57-25.05 mg TE g^{-1} aralığında, serbest radikali giderme aktivitesi (DPPH) ise sırasıyla 2.89-8.89 mg TE g^{-1}, 5.46-10.57 mg TE g^{-1}, 6.33-12.13 mg TE g^{-1} aralığında tespit edilmiştir. Araştırma sonucunda, salamura asma yapraklarının fenolik bileşikler ve antioksidan aktivite açısından zengin bir kaynak olduğu fakat tuz değerlerinin tüketim için çok yüksek seviyede olduğu belirlenmiştir. Salamura asma yapraklardan etanol ve metanol kullanılarak elde edilen ekstraktların toplam fenolik madde ve antioksidan aktivite sonuçları birbirine yakın olmakla birlikte saf su kullanılarak elde edilen sonuçlardan daha yüksektir.

Anahtar Kelimeler: Antioksidan aktivite, Narince, Asma yaprağı, Fenolik bileşik, Fizikokimyasal özellik

Introduction

The using of functional food products has been increasing in the healthy eating model (Akin-Başçam, 2021). Functional foods are defined as foods containing or enriched with nutritional components such as vitamins, minerals and various bioactive compounds which are protecting from diseases, and increase the quality of life (Granato et al., 2020). Grape is considered a functional food with its bioactive content. Grape by-products attract attention due to their nutritional and functional compounds (Akin-Başçam, 2021). The nutritional and functional value of grapes and their derivatives are found, along with their potential for financial gain. Grapes have been employed in medicine to treat or prevent diseases such as gastroenteritis, diarrhea, nausea and skin disorders since centuries ago (Lacerda et al., 2016). Apart from the grape, studies have shown that grape by products such as seed, skin and leaf also present therapeutic effects (Silva et al., 2021). In pharmacological studies, vine leaf has been found many biological activities such as antioxidant (Amarowicz et al., 2008), antimicrobial (Ceyhan et al., 2012), antihypercholesterolemic (Devi and Singh, 2017) and neuroprotective (Dani et al., 2010). In traditional medicine, grape leaves can be used in the treatment of various conditions such as bleeding, hypertension, diarrhea, eye infections, diabetes and circulatory system disorders (Akin-Basçam, 2021). Numerous effects are believed to be brought on by phenolic chemicals contained in vine leaves (Khan et al., 2021). Vine leaves contain several types of phenolic compounds such as flavonoids, tannins, anthocyanins and procyanidins (Dani et al., 2010). It is known that the beneficial effects of the antioxidant activity of phenolic compounds are due to their ability to remove oxygen and delay lipid oxidation (Zhang et al., 2021). In addition to its biological activities, the vine leaf has even outgrown the grape commercially in some regions. An important part of the pickled leaves, which are exported from Türkiye and consumed in the domestic market, are produced in Tokat and Manisa regions (Cangi and Yağcı, 2012). Tokat takes the first place in Türkiye in the production of brine leaves. The structural and sensory character of the pickled vine leaf product from Tokat has made this product important in the domestic and foreign markets (Bal et al., 2019).

Production of pickled vine leaves by fermentation method is a storage method that has been used for many years. Pickled vine leaves are fermented products that are obtained as a result of the use of organic substances, primarily carbohydrates, by microorganisms. In the brine method, the fresh leaves are brined in salt water and subjected to fermentation. Then it is packaged and presented for consumption (Sat et al., 2002; Gülcü and Torçuk, 2016). Many delicious products of Tokat cuisine, which is rich in gastronomy and has a wide variety of options, are produced using pickled vine leaves (Yaylacı and Mertol, 2021).

In the literature, there is no comprehensive study available on the evaluation of physicochemical properties, total phenolic compounds and antioxidant activity on the pickled vine leaves. In this research, it was aimed to determine some physicochemical properties, total phenolic compounds and antioxidant activity of pickled vine leaves, which are traditionally and commercially produced from the leaves of the Narince grape variety in Tokat province in the Central Black Sea Region of Türkiye.

Material and Methods

Plant material

In the research, pickled vine leaves produced by fermenting the leaves of the Narince grape variety grown in the Tokat region were used. Traditional and industrially produced pickled vine leaves samples (15 samples) were obtained. While industrially produced (12 samples) vine leaves belonging to different brands were obtained from the Merkez district (Tokat province), traditionally produced (3 samples) vine leaves were obtained from Erbaa and Niksar districts (Tokat province). All samples were collected from the market in June 2020. Because the vine leaves that are harvested and fermented for the first time in the year are release to the market in this month. For each brand, two samples of one kilogram each with the same production dates and batch numbers were obtained. Selection criteria for pickled vine leaves were based on the Narince variety grown in the Tokat region in 2020. Because the pickled vine leaves of the Narince variety, which are grown and processed in Tokat, have a significant market share in Türkiye. After the samples were obtained, they were stored at 4-8 °C under refrigerator conditions in the laboratory of the Department of Food Engineering of Tokat Gaziosmanpaşa University. The brine leaves samples produced industrially are expressed with the "E" code, and the traditionally produced samples with the "G" code.

Chemical materials

Gallic acid, trolox, ethanol, methanol, ABTS and DPPH were supplied from Sigma-Aldrich (Germany). Silver nitrate ($AgNO_3$), Folin-Ciocalteu reagent, potassium peroxydisulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), potassium chromate (K_2CrO_4), phenolphthalein, sodium hydroxide (NaOH) were supplied from Merck (Darmstadt, Germany).

Physicochemical analysis

The pH, titration acidity, salt content and color analysis (L*, a*, b*) of samples were carried out according to the method applied by Cemeroğlu (2013).

Total phenolic compounds

Phenolic compound extraction from pickled vine leaves was carried out using different solvents (distilled water, ethanol (80%) and methanol (70%). Solvent concentrations were determined by preliminary experiments. Pickled vine leaves were ground. Then, 1 g of ground leaves was extracted with 50 ml of solvent at room temperature for 24 hours. The extract was then filtered and used for analysis. 100 µL of the prepared extracts were taken and 200 µL of Folin-Ciocalteu reagent and 2 mL of distilled water were added to it and left for 3 minutes at room temperature. At the end of the period, 1 mL of sodium carbonate (Na_2CO_3) solution (20%) was added to the mixture and mixed with vortex (Velp Scientifica, Italy). A spectrophotometer (PG Instrument, T80+, England) was used to measure the mixture at 765 nm after it had been incubated for one hour at room temperature. A calibration curve was created with different concentrations of gallic acid used as standard (0, 50, 100, 150, 250, 350, 500 mg L^{-1}). Total phenolic compounds values were represented as mg gallic acid equivalent (GAE) g^{-1} (Topuz and Bayram, 2022).

Antioxidant activity

Cation radical scavenging activity ($ABTS^{\bullet+}$)

7 mM ABTS and 2.45 mM $K_2S_2O_8$ solution were prepared and mixed 1:1 for ABTS stock solution. The prepared solution was kept at room temperature in the dark for 16 hours. 40 µL of the extracts (distilled water, 80% ethanol and 70% methanol) were taken and 4 mL ABTS was added to it and left in the dark at room temperature for 6 minutes. Mixture absorbance was measured in a spectrophotometer at 734 nm. ABTS values were represented as mg trolox equivalent (TE) g^{-1} (Re et al., 1999).

Free radical scavenging activity ($DPPH^{\bullet}$)

100 µL of the prepared extracts (distilled water, 80% ethanol and 70% methanol) was taken and 3.9 mL of the prepared DPPH solution (0.06 mM) was added. It was then mixed by vortex and kept in the dark for 30 minutes. Mixture absorbance was measured in a spectrophotometer at 517 nm. DPPH values were e represented as mg trolox

equivalent (TE) g^{-1} (Blasi et al., 2016).

Statistical analysis

Statistical analyses were done by using the Duncan test through the instrument of SPSS 22.0 (IBM, USA) statistical package program. Moreover, correlation coefficients were identified using the same program.

Results and Discussions

pH Values, Total Acidity and Salt Contents

The pH values of pickled vine leaves were detected in the range of 3.31-3.95 and the average pH value was 3.60 (Table 1).

Table 1. pH values, total acidity and salt contents of pickled vine leaves

Sample*	**pH**	**Total acidity (%)***	**Salt (%)**
E1	3.83±0.01^{b}	0.56±0.05^{h}	15.83±0.07^{h}
E2	3.47±0.01^{g}	1.28±0.05^{c}	16.19±0.18^{g}
E3	3.95±0.01^{a}	0.48±0.05^{h}	10.92±0.07^{m}
E4	3.83±0.01^{b}	0.30±0.05^{i}	13.81±0.23^{j}
E5	3.71±0.01^{c}	0.56±0.05^{h}	15.44±0.12^{i}
E6	3.71±0.01^{c}	0.74±0.10^{g}	18.33±0.07^{d}
E7	3.56±0.01^{f}	0.68±0.05^{g}	12.79±0.14^{k}
E8	3.65±0.01^{d}	0.86±0.05^{f}	17.00±0.07^{f}
E9	3.48±0.01^{g}	1.16±0.09^{d}	17.36±0.18^{e}
E10	3.63±0.01^{e}	0.30±0.05^{i}	16.77±0.14^{f}
E11	3.31±0.01^{i}	0.98±0.09^{e}	23.67±0.07^{a}
E12	3.43±0.01^{h}	1.01±0.05^{e}	13.77±0.18^{j}
G1	3.54±0.01^{f}	1.72±0.05^{a}	16.89±0.18^{f}
G2	3.46±0.01^{g}	1.57±0.05^{b}	22.97±0.18^{b}
G3	3.42±0.01^{h}	1.49±0.10^{b}	22.43±0.18^{c}
Mean	3.60	0.92	16.94

Results are given as mean ± standard deviation.
* In terms of lactic acid
**Samples coded "E" and "G" represent industrial and traditional production, respectively.

The total acidity values of the pickled vine leaves were found between 0.30-1.72%. The average total acidity value is 0.92%. It was determined that the total acidity value of the brine leaves produced by the traditional method (G1, G2, G3) is higher. The salt content of pickled vine leaves was found in a wide range of 10.92-23.67%. The average value of salt content is 16.94%. Lactic acid bacteria break down carbohydrates in the media and convert sugar into lactic acid or lactic acid, CO_2, acetic acid and ethanol according to their homofermentative and heterofermentative properties (Bintsis, 2018). The main antimicrobial effect of lactic acid bacteria is the production of lactic acid and the resulting decrease in the pH value of the media (Alakomi et al., 2000). As a result of fermentation in vine leaves, with the increase in lactic acid in the media, total acidity increased, and pH value decreased.

In the study, pH values of pickled vine leaves were close to each other. The total acidity amounts of the samples were found to be higher in the traditionally produced pickled vine leaves. Generally, a decrease in pH values is observed in parallel with the titratable acidity values. But this case is not the same in all analyzed samples. There is no direct or predictable relationship between pH and titration acidity. The pH values of foods with the same titratable acidity value may differ from each other. The pH value does not depend on the concentration of acids present. However, it is affected by their dissociate abilities (AWRI, 2024).

Salt amounts of pickled vine leaves do not differ according to industrial or traditional methods. Although the salt values in the pickled vine leaves were in a wide range, a high level of salt was detected in the leaves. It is thought that this case may pose a risk in terms of health. In a study in the literature, vine leaves of different varieties were processed into canning using 1.5%, 3.5% and 5% salt (Göktürk et al., 1997), while Sultani variety vine leaves were processed into brine using 5% salt in another study (İç and Denli, 1997). It was determined that the pH, titratable acidity and salt values of the products obtained from the market are different from each other. This difference is not only between industrial and traditional production. Industrial and traditional production results also differ within themselves. Because the production process of pickled vine leaves may differ between brands. Therefore, standard production conditions should be established for the production of brine vine leaves.

Color Value

The L* value in a colorimeter symbolizes the transition from black to white between 0 and 100. The a* value determined in the colorimeter represents (+) redness and (-) greenness. The b* value specified in the colorimeter symbolizes (+) yellowness and (-) blueness (Cemeroğlu, 2013). As a result of color measurement, L* value (35.66)-(45.60), a* value (-4.38)-(-1.68), b* value (32.61)-(54.82) were determined. The color changes from green to yellow as a result of the fermentation of the vine leaves. The E4 sample had the highest value in terms of L* (brightness) and b* (yellowness) characteristics, while sample E2 was the highest value in terms of a* (greenness) characteristics (Table 2).

Although there are many derivatives of chlorophyll, the most important ones are chlorophyll a and chlorophyll b. Their most important duty is to provide the conversion of solar energy into chemical energy during photosynthesis in the plant. Solar rays are absorbed by chlorophyll a and chlorophyll b at different wavelengths, and the two chlorophylls complement each other. In the spectral area between 500-600 nm, very little light is absorbed and most of it is reflected, so plants appear green (Ergün, 2003). Chlorophyll pigments are broken down during processing (heat, light, acid, etc.) applied to foods and color changes are observed in the plant with the effect of fermentation, the lactic acid in the media increases. Thus, chlorophyll, which gives the leaf its color, is destroyed by the effect of acid. As a result, chlorophyll is converted to pheophytin and pheophorbides. As the leaves lose their green color, a color change is observed from mat green to olive yellow instead of bright green (Kazancı, 2008).

Table 2. Color values of pickled vine leaves

Sample***	L*	a*	b*
E1	43.09±0.80^{e**}	-3.07±0.25^{cd}	41.19±1.10^{cd}
E2	43.20±0.86^{e}	-1.68±0.40^{a}	42.20±0.40^{c}
E3	40.02±0.70^{g}	-2.25±0.10^{b}	32.61±1.39^{g}
E4	50.67±0.54^{a}	-2.82±0.06^{c}	54.82±1.28^{a}
E5	42.61±0.31^{e}	-2.88±0.11^{c}	39.95±0.43^{de}
E6	40.27±0.59^{g}	-2.30±0.11^{b}	35.54±1.27^{f}
E7	41.30±0.26^{f}	-3.34±0.05^{d}	45.90±1.03^{b}
E8	35.66±0.72^{i}	-2.80±0.27^{c}	35.62±1.04^{f}
E9	44.24±0.31^{d}	-4.53±0.17^{h}	40.81±0.62^{cde}
E10	37.29±0.30^{h}	-2.23±0.05^{b}	37.46±0.41^{f}
E11	45.60±0.11^{b}	-4.02±0.03^{f}	39.24±0.71^{e}
E12	44.48±0.28^{cd}	-4.47±0.01^{gh}	40.10±0.08^{de}
G1	50.22±0.46^{a}	-4.20±0.09^{fg}	45.63±1.05^{b}
G2	43.22±0.16^{e}	-3.73±0.13^{e}	36.03±0.70^{f}
G3	45.19±0.15^{bc}	-4.38±0.03^{gh}	42.25±0.42^{c}

Results are given as mean ± standard deviation.
** Small letters in the same column indicate statistical difference between leaf varieties. ($P<0.05$)
*** Samples coded "E" and "G" represent industrial and traditional production, respectively.

It is observed that there is a difference in the color values of pickled vine leaves obtained from the market. Although the pickled vine leaf samples belong to the same variety (Narince) and the same season (June 2020), it is possible that there are differences between the color values of samples. Because the processing parameters (salt, temperature, packaging, etc.) to brine also vary from brand to brand, in addition to the growing and harvesting conditions of the leaves. Therefore, it is possible to detect differences between the L*, a* and b* color values of the samples.

Total Phenolic Compounds

Grapes contain phenolic compounds such as flavanols and anthocyanins. Anthocyanins and flavanols are found in grape skins and leaves, whereas proanthocyanidins and other non-flavonoid substances are primarily found in the pulp and seeds (Di Lorenzo et al., 2019). According to a study, the highest levels of total phenolic compounds were discovered in seeds followed by leaves, while the lowest levels were discovered in skin and pulp (Šuković et al., 2020). According to studies, grape leaves are an important source of phenolic compounds (Labanca et al., 2020).

The gallic acid standard calibration curve ($y=0.0028x+0.0836$, $R^2=0.99$) is created to calculate total phenolic. The total phenolic compounds amount of the distilled water extract was determined to be lower than ethanol and methanol extract. The total phenolic compounds in the extracts obtained using distilled water were determined in the range of 0.55-7.81 mg GAE g^{-1}. The total phenolic compounds amount of the samples using ethanol or methanol was determined close to each other. The highest amount of phenolic compounds for both solvents was in the G2 pickled vine leaves. The amount of total phenolic compound was determined as 3.98-15.66 mg GAE g^{-1} in ethanol extracts and 3.49-16.85 mg GAE g^{-1} in methanol extracts (Table 3).

When the total phenolic compounds data were evaluated, it was determined that the amounts of phenolic compounds changed according to the type of solvent. The type of solvent to be used in the extraction of phenolic compounds is very important (Çoklar and Akbulut, 2016). The solvent system used in the extraction stage affects the profile of the phenolic compounds as well as the amounts of total phenolic compounds extracted from the plant material (Türkyılmaz et al., 2017).

While the high amount of total phenolic compounds was detected in the 80% ethanol extracts in some samples, the high amount of total phenolic compounds was detected in the 70% methanol extracts in the other samples. Because the solubility of phenolics may vary depending on the type of solvent used, the degree of polymerization of phenolics and the formation of insoluble complexes with other components (Yolci et al., 2022).

Table 3. The total phenolic compound of pickled vine leaves (mg GAE g^{-1})

Sample**	Water Extract	80% ethanol Extract	70% methanol Extract
E1	$1.26{\pm}0.13^{Bj*}$	$5.00{\pm}0.57^{Afgh}$	$5.28{\pm}0.16^{Ai}$
E2	$2.85{\pm}0.25^{Bf}$	$6.23{\pm}0.39^{Ae}$	$6.15{\pm}0.40^{Agh}$
E3	$0.55{\pm}0.06^{Ck}$	$3.98{\pm}0.16^{Ah}$	$3.49{\pm}0.03^{Bj}$
E4	$1.55{\pm}0.16^{Cij}$	$4.90{\pm}0.05^{Bgh}$	$5.78{\pm}0.43^{Ahi}$
E5	$1.51{\pm}0.13^{Bij}$	$5.28{\pm}0.40^{Aefg}$	$5.16{\pm}0.04^{Ai}$
E6	$2.12{\pm}0.09^{Cgh}$	$6.17{\pm}0.03^{Bef}$	$6.86{\pm}0.27^{Ag}$
E7	$2.24{\pm}0.03^{Bg}$	$5.99{\pm}0.05^{Aefg}$	$6.54{\pm}0.45^{Agh}$
E8	$3.25{\pm}0.09^{Ce}$	$9.00{\pm}0.24^{Ac}$	$7.87{\pm}0.11^{Bf}$
E9	$7.03{\pm}0.14^{Bb}$	$12.38{\pm}1.04^{Ab}$	$12.69{\pm}0.56^{Ac}$
E10	$1.76{\pm}0.08^{Bhi}$	$5.69{\pm}0.11^{Aefg}$	$5.26{\pm}0.40^{Ai}$
E11	$3.82{\pm}0.32^{Bd}$	$7.69{\pm}0.59^{Ad}$	$8.88{\pm}0.48^{Ae}$
E12	$5.97{\pm}0.05^{Bc}$	$12.19{\pm}0.68^{Ab}$	$11.11{\pm}0.53^{Ad}$
G1	$7.60{\pm}0.18^{Ba}$	$14.54{\pm}0.40^{Aa}$	$15.33{\pm}0.35^{Ab}$
G2	$5.71{\pm}0.37^{Bc}$	$15.66{\pm}0.59^{Aa}$	$16.85{\pm}0.45^{Aa}$
G3	$7.81{\pm}0.30^{Ca}$	$15.15{\pm}0.91^{Aa}$	$12.15{\pm}0.25^{Bc}$

Results are given as mean ± standard deviation.
* Small letters in the same column indicate the difference between samples, and capital letters in the same line indicate the difference between solvent extracts ($P<0.05$).
**Samples coded "E" and "G" represent industrial and traditional production, respectively.

In a study, total phenolic compound of pickled vine leaves of Narince Bağ, Narince Yerli, Narince Aşılı, Sultani Çekirdeksiz Aşılı were determined as 8.00, 11.56, 9.96, 10.34 mg GAE g^{-1}, respectively (Semerci, 2019). In another study, the total phenolic compound of fresh vine leaves grown under optimum conditions and drought stress were determined as 19.37 and 15.94 mg GAE g^{-1}, respectively (Król et al., 2014). The amounts of total phenolic compound obtained as a result of this study are very close to the values determined in fresh and pickled vine leaves in the literature. Kosar et al. (2007) stated that the total phenolic compound levels in leaves are not affected by the brining method. According to the literature, the total phenolic content of leaves gathered from various grape cultivars was found to vary by cultivar, and grape leaves had high phenolic compounds similar to berries. This showed that grape leaves are a rich source of phenolic and antioxidant compounds (Babalık and Baydar, 2019). The total phenolic compound of vine leaves from Sultani Çekirdeksiz, Sultan 1, Sultan 7, Saruhanbey, and Narince grape cultivars were found between 9.72 and 14.22 mg GAE g^{-1} (Güler and Candemir, 2014). Banjanin et al. (2021) researched the effect of grape varieties on total phenolic compounds amounts of vine leaves. Total phenolic compounds of vine leaves were determined between 12.98 mg GAE g^{-1} and 17.48 mg GAE g^{-1}.

Antioxidant activity of pickled vine leaves

The cation radical scavenging activity of the distilled water extract was determined to be lower than ethanol and methanol extract (Table 4). The trolox standard calibration curve ($y=-0.001x+0.6344$, $R^2=0.99$) is created to calculate cation radical scavenging activity. When antioxidant activity is calculated using the calibration curve equation, cation radical scavenging activity in the extracts obtained using distilled water was found between 1.87-15.42 mg TE g^{-1}. The cation radical scavenging activity of the samples using ethanol or methanol was determined close to each other. Cation radical scavenging activity was determined in the range of 2.75-24.85 mg TE g^{-1} in ethanol extracts, and in the range of 3.57-25.05 mg TE g^{-1} in methanol extracts. The lowest cation radical scavenging activity was determined in water extracts. Although 80% ethanol and 70% methanol extracts are close to each other, it is not possible to determine which is best for the two solvents. The reason for obtaining different values for both solvents in the samples may be the composition and amount of compounds with antioxidant activity, such as phenolic compounds.

In a study, ABTS values of 8 vine leaf (immature and mature) varieties harvested in June and September varied between 311.59-715.85 µmol TE g^{-1} (Gülcü et al., 2020). ABTS values of the vine leaves of the Gohér variety harvested from the sun and shaded areas ranged from 0.65 to 1.88 µM TE g^{-1} (Bodó et al., 2017).

Table 4. Antioxidant activity of pickled vine leaves

Sample**	Cation radical scavenging activity (mg TE g^{-1})			Free radical scavenging activity (mg TE g^{-1})		
	Distilled water	80% ethanol	70% methanol	Distilled water	80% ethanol	70% methanol
E1	2.82±0.42^{Aef*}	4.80±0.32^{Agh}	5.67±1.77^{Agh}	3.60±0.35^{Bg*}	5.69±0.06^{Ag}	6.81±0.49^{Agh}
E2	3.02±0.78^{Cdef}	12.02±0.64^{Ae}	8.15±0.95^{Bef}	4.07±0.55^{Bfg}	6.56±0.02^{Aef}	7.32±0.27^{Ag}
E3	2.25±0.18^{Bg}	2.75±0.04^{Ah}	3.57±0.42^{Ah}	2.89±0.18^{Bh}	6.96±0.51^{Ae}	6.33±0.45^{Ah}
E4	1.95±0.39^{Cfg}	5.27±0.28^{Bg}	8.00±0.60^{Aef}	4.39±0.06^{Cef}	8.26±0.20^{Bd}	9.15±0.04^{Ade}
E5	2.17±0.21^{Bf}	4.95±0.95^{Bg}	8.30±1.52^{Aef}	4.58±0.06^{Cef}	6.04±0.16^{Bfg}	7.01±0.39^{Ag}
E6	4.25±0.74^{Bde}	7.6±0.67^{ABf}	10.90±1.66^{Ad}	4.60±0.59^{Cef}	7.26±0.04^{Be}	8.57±0.20^{Aef}
E7	3.72±1.06^{Adef}	7.8±2.65^{Af}	8.42±0.07^{Ae}	4.33±0.06^{Bef}	6.56±0.33^{Aef}	7.24±0.27^{Ag}
E8	4.65±0.88^{Bde}	9.42±1.13^{Af}	11.37±0.28^{Ad}	4.97±0.29^{Cde}	7.06±0.41^{Be}	8.19±0,14^{Af}
E9	12.42±0.35^{Bb}	16.95±1.03^{Ad}	18.15±0.25^{Ac}	3.93±0.08^{Bfg}	9.65±0.24^{Abc}	10.46±0.39^{Ab}
E10	1.87±0.14^{Bfg}	7.75±0.39^{Af}	5.95±1.10^{Afg}	7.85±0.51^{Bb}	5.46±0.31^{Ag}	7.18±0.27^{Ag}
E11	4.82±0.14^{Bd}	12.10±0.67^{Ae}	11.40±0.53^{Ad}	5.36±0.02^{Cd}	7.14±0.10^{Be}	8.57±0.47^{Aef}
E12	9.55±0.39^{Cc}	20.52±1.20^{Bc}	25.05±1.38^{Aa}	7.39±0.06^{Cb}	10.19±0.14^{Aab}	9.76±0.04^{Bcd}
G1	12.02±1.34^{Bb}	22.45±0.25^{Abc}	22.07±1.06^{Ab}	8.89±0.06^{Ca}	10.57±0.20^{Ba}	12.13±0.04^{Aa}
G2	15.42±1.56^{Ba}	24.85±0.53^{Aa}	22.17±0.85^{Ab}	8.60±0.47^{Ba}	9.43±0.31^{Bc}	11.71±0.08^{Aa}
G3	9.22±1.48^{Cc}	24.32±0.71^{Aab}	17.35±0.95^{Bc}	6.42±0.33^{Bc}	9.60±0.67^{Abc}	10.17±0.22^{Abc}

Results are given as mean ± standard deviation.
* Small letters in the same column indicate the difference between examples, and capital letters in the same line indicate the difference between solvent extracts (P<0.05).
** Samples coded "E" and "G" represent industrial and traditional production, respectively.

The free radical scavenging activity of pickled vine leaves is given in Table 4. The trolox standard calibration curve ($y=-0.0018x+0.5265$, $R^2=0.99$) is created to calculate free radical scavenging activity. When antioxidant activity is calculated using the calibration curve equation, free radical scavenging activity in the extracts obtained using distilled water was found between 2.89-8.89 mg TE g^{-1}. Free radical scavenging activity was determined in the range of 5.46-10.57 mg TE g^{-1} in 80% ethanol extracts and 6.33-12.13 mg TE g^{-1} in 70% methanol extracts. The highest free radical scavenging activity in all three solvents was determined in the G1 sample.

Free radical scavenging analysis was carried out in the extracts obtained using distilled water, 80%

ethanol and 70% methanol. The free radical scavenging activity of the distilled water extract (except for E10 sample) was determined to be lower than ethanol and methanol extract. This may be due to the individual phenolic compound content of the pickled vine leaf of sample E10 or other antioxidant compounds in its content.

In a study, it was determined that the free radical scavenging activity of 8 vine leaf (immature and mature) varieties harvested in June and September varied between 14.54-30.24 µmol TE g^{-1} (Gülcü et al., 2020). In another study, it was determined that the DPPH activity of the extracts obtained from vine leaves using 8 different solvents varied between 714.71-6496.99 mg TE g^{-1} extract (Matloub, 2018). In a study, fresh vine leaf showed higher antioxidant activity as well as total phenolic compounds than frozen and canned vine leaf. This result was associated with the preservation method. These effects may induce the breakdown of antioxidant compounds or the destruction of the active metabolites (Jaradat et al., 2017).

Correlation coefficients between total phenolic compound and cation radical scavenging activity (ABTS), total phenolic compound and free radical scavenging activity (DPPH), cation radical scavenging activity (ABTS) and free radical scavenging activity (DPPH) are given in Table 5. It was determined that there was an inverse relationship between total phenolic compounds and ABTS, and between ABTS and DPPH in the E10 sample. A positive correlation was found between total phenolic compound and ABTS, total phenolic compounds and DPPH, and ABTS and DPPH in all samples except E10. In general, there is a relationship between phenolic compounds and antioxidant activity. Because phenolic compounds were considered to have antioxidant activities due to their behavior such as singlet oxygen quenchers, reducing agents, and hydrogen donor (Özer et al., 2018). There is a negative correlation between total phenolic compounds and DPPH in the E10 sample. This may be associated with the low free radical scavenging activity of the phenolic compound profile in the E10 sample.

Table 5. Correlation coefficients

	Correlation coefficients		
Sample*	**Total phenolic compound and ABTS**	**Total phenolic compound and DPPH**	**ABTS and DPPH**
E1	0.824	0.931	0.770
E2	0.877	0.939	0.741
E3	0.922	0.991	0.894
E4	0.959	0.853	0.753
E5	0.796	0.885	0.884
E6	0.903	0.969	0.936
E7	0.872	0.972	0.913
E8	0.865	0.939	0.971
E9	0.978	0.969	0.974
E10	0.971	-0.735	-0.838
E11	0.919	0.966	0.827
E12	0.902	0.987	0.900
G1	0.985	0.917	0.848
G2	0.907	0.769	0.444
G3	0.993	0.840	0.789

* Samples coded "E" and "G" represent industrial and traditional production, respectively.

Conclusion

In this present study, the pH values of the pickled vine leaves were close to each other. The total acidity amounts of the samples were found to be higher in the traditionally produced pickled vine leaves. Salt amounts of pickled vine leaves do not differ according to industrial or traditional methods. Although the salt values in the pickled vine leaves were in a wide range, a high level of salt was detected in the leaves. It is thought that this situation may pose a risk in terms of health. For total phenolic compounds and antioxidant activities (distilled water, 80% ethanol, 70%

methanol), it was found that the results obtained using 80% ethanol and 70% methanol were close to each other and total phenolic compounds and antioxidant activities of these extracts are higher than water extracts.

When the obtained data is evaluated, due to the lack of certain standards in the production of pickled vine leaves, serious differences were observed in quality parameters such as salt content. Determination of standard production parameters to produce of pickled vine leaves is important in terms of establishing a reliable market. The results obtained are important in terms of being a comprehensive study of the quality characteristics of Narince pickled vine leaves and being a source for future research and studies.

Acknowledgments

This study was financially supported by Tokat Gaziosmanpaşa University Scientific Research Projects Unit (TOGU-BAP) under project number 2019/19. This paper is a part of MSc thesis written by Tuba Zorlu Ünlü.

Conflict of interest

The authors claim no conflict of interest.

Author contributions

Tuba Zorlu Ünlü: Sample collection, Analysis, Writing original manuscript. **Semra Topuz:** Methodology, Evaluation, Writing original manuscript, Reviewing & editing. **Mustafa Bayram:** Supervision, Evaluation, Writing-review & editing. **Cemal Kaya:** Writing-review & editing.

References

Akin-Basçam, Z. (2021). Functional composition of grapevine leaf and required procedures for the design of the brine grapevine leaf processing plant. *International Journal of Scientific and Technological Research, 7*(1), *1-14*.

Alakomi, H. L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, *66*(5), 2001-2005.

Amarowicz, R., Narolewska, O., Karamac, M., Kosinska, A., & Weidner, S. (2008). Grapevine leaves as a source of natural antioxidants. *Polish Journal of Food and Nutrition Sciences*, *58*(1), 73-78.

AWRI, (2024). Australian Wine Research Institute. Retrieved from: https://awri.com.au.

Babalık, Z., & Baydar, N. G. (2019). Grape leaves as a natural antioxidant source: Determination of total phenolic compound changes. *Turkish Journal of Agriculture-Food Science and Technology*, *7*(10), 1603-1606.

Bal, H. S. G., Kaplan, E., & Kaplan, K. (2019). Bağcılık ve salamura yaprak konusunda çiftçi yönelimi ve değerlendirmeleri (Tokat ili Pazar ilçe örneği). 3. *Uluslararası UNİDOKAP Karadeniz Sempozyumu*, (pp 849-857), 21-23 Haziran, Tokat, Türkiye.

Banjanin, T., Uslu, N., Vasic, Z. R., & Özcan, M. M. (2021). Effect of grape varieties on bioactive properties, phenolic composition, and mineral contents of different grape-vine leaves. *Journal of Food Processing and Preservation*, *45*(2), e15159.

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiology*, *4*(4), 665-684.

Blasi, F., Urbani, E., Simonetti, M. S., Chiesi, C., & Cossignani, L. (2016). Seasonal variations in antioxidant compounds of *Olea europaea* leaves collected from different Italian cultivars. *Journal of Applied Botany and Food Quality*, *89*, 202-207.

Bodó, A., Csepregi, K., Szata, B. É., Nagy, D. U., Jakab, G., & Kocsis, M. (2017). Bioactivity of leaves, skins and seeds of berry color variant grapevines (*Vitis vinifera* L.). *Research & Reviews: Journal of Pharmacognosy and Phytochemistry*, 16-22.

Cangi, R., & Yağcı, A. (2012). Iğdır yöresinde salamuralık asma yaprağı üretim imkanları. *Iğdır University Journal of the Institute of Science and Technology*, *2*, 9-14.

Cemeroğlu, B. S. (2013). Gıda Analizleri, 3th ed. Bizim Grup Basımevi, Ankara.

Ceyhan, N., Keskin, D., Zorlu, Z., & Ugur, A. (2012). In-vitro antimicrobial activities of different extracts of grapevine leaves (*Vitis vinifera* L.) from West Anatolia against some pathogenic microorganisms. *Journal of Pure Applied Microbiology*, *6*, 1303-1308.

Çoklar, H. & Akbulut, M. (2016). Alıç (*Crataegus orientalis*) meyvesinin antioksidan aktivitesi ve fenolik bileşiklerinin ekstraksiyonu üzerine farklı çözgenlerin etkisi. *Derim, 33* (2), 237-248.

Dani, C., Oliboni, L. S., Agostini, F., Funchal, C., Serafini, L., Henriques, J. A., & Salvador, M. (2010). Phenolic content of grapevine leaves (*Vitis labrusca* var. Bordo) and its neuroprotective effect against peroxide damage. *Toxicology in Vitro*, *24*(1), 148-

153.

Devi, S., & Singh, R. (2017). Evaluation of antioxidant and anti-hypercholesterolemic potential of *Vitis vinifera* leaves. *Food Science and Human Wellness*, *6*(3), 131-136.

Di Lorenzo, C., Colombo, F., Biella, S., Orgiu, F., Frigerio, G., Regazzoni, L., de Sousa, L. P., Bavaresco, L., Bosso, A., Aldini, G., & Restani, P. (2019). Phenolic profile and antioxidant activity of different grape (*Vitis vinifera* L.) varieties. *BIO Web of Conferences*, *12*, 04005.

Ergün, E. (2003). Klorofil a ve klorofil b'nin türev spektrofotometrik yöntem ile tayini (Yüksek lisans tezi). İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü, İstanbul, Türkiye.

Göktürk, N., Artık, N., Yavaş, İ., & Fidan, Y. (1997). Bazı üzüm çeşitleri ve asma anacı yapraklarının yaprak konservesi olarak değerlendirilme olanakları üzerinde bir araştırma. *Gıda*, *22*(1).

Granato, D., Barba, F. J., Kovaˇcevi´c, D. B., Lorenzo, J. M., Cruz, A.G., & Putnik, P. (2020). Functional foods: Product development, technological trends, efficacy testing, and safety. *Annual Review of Food Science and Technology, 11*, 93-118.

Gülcü, M., & Torçuk, A.İ. (2016). Yemeklik asma yaprağı üretimi ve pazarlamasında kalite parametreleri. *Fruit Science*, *1*, 75-79.

Gülcü, M., Ghafoor, K., Al-Juhaimi, F., Özcan, M. M., Uslu, N., Babiker, E. E., Mohamed Ahmed, I. A., & Azmi, I. U. (2020). Effect of grape (*Vitis vinifera* L.) varieties and harvest periods on bioactive compounds, antioxidant activity, phenolic composition, mineral contents, and fatty acid compositions of Vitis leave and oils. *Journal of Food Processing and Preservation*, *44*(11), e14890.

Güler, A., & Candemir, A. (2014). Total phenolic and flavonoid contents, phenolic compositions and color properties of fresh grape leaves. *Türk Tarım ve Doğa Bilimleri Dergisi*, *1*(Özel Sayı-1), 778-782.

İç, E., & Denli, Y. (1997). Sultani asma yapraklarından salamura yaprak üretimi. *Gıda*, *22*(2).

Jaradat, N. A., Zaid, A. N., Hussen, F., & İyad, A. (2017). The effects of preservation methods of grapevine leaves on total phenols, total flavonoids and antioxidant activity. *Marmara Pharmaceutical Journal*, *21*(2), 291-297.

Kazancı, Y. T. (2008). Hıyar turşusu üretiminde farklı asit ve pH'nın renk stabilitesi üzerine etkisi (Yüksek lisans tezi). Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Bursa.

Khan, Y., Khan, S. M., ul Haq, I., Farzana, F., Abdullah, A., Abbasi, A. M., Alamri, S., Hashem, M., Sakhi, S., Asif, M., & Shah, H. (2021). Antioxidant potential in the leaves of grape varieties (*Vitis vinifera* L.) grown in different soil compositions. *Arabian Journal of Chemistry*, *14*(11), 103412.

Koşar, M., Küpeli, E., Malyer, H., Uylaşer, V., Türkben, C., & Başer, K. H. C. (2007). Effect of brining on biological activity of leaves of *Vitis vinifera* L. (Cv. Sultani Cekirdeksiz) from Turkey. *Journal of Agricultural and Food Chemistry*, *55*(11), 4596-4603.

Król, A., Amarowicz, R., & Weidner, S. (2014). Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta Physiologiae Plantarum*, *36*, 1491-1499.

Labanca, F., Faraone, I., Nolè, M. R., Hornedo-Ortega, R., Russo, D., García-Parrilla, M. C., Chiummiento, L., Bonomo, M. G., & Milella, L. (2020). New ınsights into the exploitation of *Vitis vinifera* L. cv. Aglianico leaf extracts for nutraceutical purposes. *Antioxidants*, *9*(8), 708.

Lacerda, D. S., Costa, P. C., Funchal, C., Dani, C., & Gomez, R. (2016). Benefits of vine leaf on different biological systems. *Grape and Wine Biotechnology*, *19*, 125-143.

Matloub, A. A. (2018). Optimization of polyphenol extraction from *Vitis vinifera* L. leaves, antioxidant activity and its correlation with amelioration effect on AlCl3-induced Alzheimer's disease. *Archives of Pharmaceutical Sciences Ain Shams University*, *2*(2), 97-110.

Özer, Z., Kılıç, T., Çarıkçı, S., & Yılmaz, H. (2018). Investigation of phenolic compounds and antioxidant activity of Teucrium polium L. decoction and infusion. *Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *20*(1), 212-218.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231-1237.

Sat, I. G., Sengul, M., & Keles, F. (2002). Use of grape leaves in canned food. *Pakistan Journal of Nutrition*, *1*(6), 257-262.

Semerci, G. (2019). Asma fidanı yapraklarının salamuralık olarak değerlendirilme imkânları üzerine bir araştırma (Yüksek lisans tezi). Tokat Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Tokat.

Silva, A., Silva, V., Igrejas, G., Gaivão, I., Aires, A., Klibi, N., Enes Dapkevicius, M. d. L., Valentão, P., Falco, V., & Poeta, P. (2021). Valorization of winemaking by-products as a novel source of antibacterial properties: New strategies to fight antibiotic resistance. *Molecules*, *26*(8), 2331.

Šuković, D., Knežević, B., Gašić, U., Sredojević, M., Ćirić, I., Todić, S., Mutić, J., & Tešić, Ž. (2020). Phenolic profiles of leaves, grapes and wine of grapevine variety vranac (*Vitis vinifera* L.) from Montenegro. *Foods*, *9*(2), 138.

Topuz, S., & Bayram, M. (2022). Oleuropein extraction from leaves of three olive varieties (*Olea europaea* L.): Antioxidant and antimicrobial properties of purified oleuropein and oleuropein extracts. *Journal of Food Processing and Preservation*, *46*(6), e15697.

Türkyılmaz, M., Tağı, Ş., & Özkan, M. (2017). Narın farklı bölümlerinin polifenol içeriği, antioksidan ve antibakteriyel aktivitesi üzerine ekstraksiyon çözgenlerinin etkisi. *Akademik Gıda*, *15*(2), 109-118.

Yaylacı, S., & Mertol, H. (2021). Coğrafi işaretli ürünler ve gastronomik lezzetler: Tokat örneği. *Ağrı İbrahim Çeçen Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*, *7*(1), 313-334.

Yolci, M. S., Tunçtürk, R., & Tunçtürk, M. (2022). Farklı ekstraksiyon çözücüleri ve hasat zamanlarının aspir (*Carthamus tinctorius* L.) çiçeklerinin toplam fenol ve flavonoid miktarları ile antioksidan aktivitesi üzerine etkileri. *International Journal of Life Sciences and Biotechnology*, *5*(1), 97-109.

Zhang, S., Willett, S. A., Hyatt, J. R., Martini, S., & Akoh, C. C. (2021). Phenolic compounds as antioxidants to improve oxidative stability of menhaden oil-based structured lipid as butterfat analog. *Food Chemistry*, *334*, 127584.