ABSTRACT

This study investigates the physicochemical, phytochemical, and mineral properties of juices are known as verjuice obtained from unripe black grapes. Moisture, pH, total soluble solids (°Brix), total titratable acidity, crude fibre and vitamin C (ascorbic acid) were found to be 74.6%, 2.3%, 3%, 4.5% and 15mg/100ml, respectively. Organic acid content (tartaric and malic) was determined as 15.2 and 7.4 mg/ml, respectively, and total phenol content was 307 mg/l. Total antioxidant capacity was found to be 36 μg/ml (Gallic acid), determined using a DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay and total anthocyanin content was 23.43 mg/l. Unripe grapes are relevant for use as an acidifying agent for extending the shelf life of foods.

Keywords: Anthocyanin, Antioxidant, Crude fibre, Organic acids, Vitamin C

How to cite this article: Shakir K, Salih Rashid RM (2019): Physiochemical and phytochemical profile of unripe black grape juice, Ann Trop Med & Public Health; 22(IV): S359. DOI: http://doi.org/10.36295/ASRO.2019.22126

INTRODUCTION

Unripe grapes, Vitis vinifera, comprise the stage prior to achieving maturation and can be either black, white or red. These grapes, which have a citrus and tart taste, are used as an acidifying agent for foods in the form of juice or powder after drying. In Kurdistan, they are known as a Latin word ‘Bersîla’, while in Persian and Turkish they are referred to as ‘Abe ghureh’ and ‘koruksuyu’, respectively. The name verjuice derives from the French ‘vert jus’ (green grape) (1). Generally, many products are processed by boiling and salting verjuice, such as a sour juices, sauces and syrups; these products have a sour taste similar to vinegar or lemon juice, and are used as appetizers and flavouring agents for salads and pickling (2,3). In Kurdish culture, verjuice is used to tenderize meat, chicken breast and fish, with some people drying the berries and converting them to a powder for use as a natural alternative to acid agents. Traditionally, verjuice is extracted by pressing green unripe grapes through a non-metallic instrument and preserved in bottles (4). Verjuice is a source of phytochemical compounds, such as flavonoids, phenolic acids and compounds of hydroxybenzoic acid, hydroxycinnamic acid, caffeic acid, caftaric acid, catechin, epicatechin, gallic acid, tartaric acid, p-coumaric acid, protocatechuic acid, quercetin and tyrosol (5). There is an inverse relationship between the use of antioxidant flavanols and cardiovascular disease (6). With respect to high and low-density lipoprotein cholesterol (HDL-C and LDL-C) although, total antioxidant capacity (TAC), and their effects. (7) showed that
drinking verjuice after eating twice per day significantly increases HDL-C and TAC. On the other hand, grapes and its products are a source of different minerals that essential for human health such a (Fe, Cu, Se and Zn) also potassium, sodium, calcium and magnesium \(^{(8,9)}\). The high concentration of minerals in the grape effect of regulating blood pressure, immune system, bone and haemoglobin components \(^{(10)}\). Therefore, the verjuice is important for human health and food processing scopes depend on for mentioned studies, so the aim of this study is to identify some physicochemical and phytochemical properties of unripe black grapes, prior to maturation as black grapes, with a view to using them in a similar manner to other fruit juice drinking.

**MATERIAL AND METHODS**

**Study area and Unripe black grape sample collection**

Unripe black grape samples (*Vitis vinifera*) (Fig.1) were harvested in summer 2017 before (*véraison*) from local vineyards in Welayar -Sulaimanya, located in Northeast Iraq Lat 35°32.22 and Long 45°32.02 (Figure 2). The climate of the study area is classified as a semi-arid region which is hot and dry in summer and cold in winter \(^{(11)}\).

![Figure 1: Unripe black grape berries](image1)

![Figure 2: Regional and local location of the study area](image2)
Production of unripe grape juice

After harvesting, clusters were soaked in drink water for 24 h to remove pesticides, dust, soil and other impurities. Stalks were discarded, and berries were passed through a crusher machine (Gosonic) then pressed. The juice obtained is filtered and stored at 2–4°C (Fig. 3).

![Diagram of the lab-processing of unripe black grape juice obtained]

**Figure 3: Lab-processing of unripe black grape juice obtained**

**Physicochemical properties**

**Determination of moisture content:**

A clean, dried crucible was weighed (W1) and 5 gm of the sample was then also weighed (W2) and placed into an oven at 70°C. After cooling in desiccators, the sample was weighed to constant weight (W3), a method described. The percentage of moisture was calculated as follows:

\[
\% \text{Moisture} = \frac{W3 - W1}{W2 - W1} \times 100
\]

**(Eq.1)**

**Determination of pH**

The pH was determined using a pH meter (Jenway) after calibration with a buffer solution.

**Determination of ash**

The (12) method was used, with a dried porcelain crucible being weighed (W1). Two grams of sample was placed into the previously weighed porcelain crucible and reweighed (W2). Samples were heated in an oven and then transferred into a muffle furnace (550°C) for eight hours. The crucible was then cooled in desiccators and weighed again (W3), with the ash percentage being calculated as.
Determination of crude fibre

Two grams of samples were weighed and placed into a round-bottom flask. One hundred ml of 0.25M sulfuric acid was added and the mixture boiled under reflux for 30 minutes. The residue was then washed using distilled water to remove acids and 100 ml of 0.3M sodium hydroxide was then added and boiled for 30 minutes, washed by distilled water and dried in an oven at 100°C for 80 minutes. After cooling in desiccators and weighing (C1), the sample was incinerated in a muffle furnace at 550°C for two hours, cooled and reweighed (C2). Finally, crude fibre was calculated.

\[
\text{crude fibre} = \frac{C2 - C1}{C1} \times 100
\]  

(Eq.3)

Determination of Titratable acidity and sugar-acid ratio

Ten ml of verjuice was added to 100 ml of deionized water, titrated with 0.1N sodium hydroxide solution, using phenolphthalein as an indicator. Total acidity and total titratable acidity (g/L) were calculated as tartaric acid equivalent weight using the following equations.

\[
\%\text{Acid} = \frac{\text{titre of base (ml)} \times \text{acid factor (0.1N \times equivalent weight)}}{10 \text{ml of sample}} \times 100
\]  

(Eq.4)

\[
\text{Sugar : Acid ratio} = \frac{\text{Brix}}{\text{Acidity}}
\]  

(Eq.5)

Determination of total soluble solids and density

The total soluble solid °Brix content of juice was determined as a Brix by using a hand refractometer (China). Two drops of juice were placed on the sample location and the scale number was read. Density was measured using a pycnometer assay.

Determination of juice content

One hundred berries of unripe grapes were selected randomly, weighed and recorded. Then, the juice was extracted using a juice maker and the weight was recorded in grams. The percentage of juice content was calculated using the following formula.

\[
\% \text{Ash} = \frac{W3 - W1}{W2 - W1} \times 100
\]  

(Eq.2)
Determination of Organic acids by HPLC

Chromatographic analysis was carried out using the Knaur HPLC system (Germany) with a triathlon autosampler and a UV-vis detector (Knaur, k-2600-wellchrom) with a k-1001 pump. Before injection, samples were centrifuged (4 min at 5000rpm), and an injection volume of 20μl was used for transferring the sample to an ultra ES-FS (250mm × 3mm × 5μm) column to separate organic acids components. The mobile phase consisted of sulfuric acid 0.0025% (w/v), with a flowrate of 0.2 ml/min, and the chromatogram being recorded at 210 nm (14).

Determination of antioxidant capacity and total phenolic

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to measure the antioxidant activity of verjuice based on the evaluation of free radical scavenging capacities of juices. A 100 μM of DPPH solution was prepared in 10ml of methanol and 2.3 ml of this solution was added to 0.5ml of verjuice in methanol at the same concentration (0.1mg/ml). After 20 minutes, incubated in a dark place at room temperature. gallic acid was used as a standard and absorbance is measured at 520 nm, then the percentage of remaining DPPH was calculated using the equation:

\[
100\% \times \frac{A_{control} - A_{sample}}{A_{control}} = DPPH \\
\]

Total phenolic content was evaluated using the Folin-Ciocalteu method with a UV-visible instrument at 660 nm; total phenolics were measured against a calibration curve in the range between (0-400)mg/l obtained with p-coumaric acid (14,15).

Determination of anthocyanin content

Two ml of juice was diluted up to 25 ml with pH 1 solution (125 ml 0.2M KCl and 375 ml 0.2M of HCl) and pH 4.5 solutions (400 ml of 1M CH₃CO₂Na. 240 ml of 1M HCl). The absorbance of the solutions was measured at 510 nm. Anthocyanin concentration was calculated using the equation.

\[
C_{mg/ml} = (AbspH1 - Abs pH4.5) \times 484.82 \times \frac{1000}{24825} \times DF \\
\]

Were 484.42 is the molecular mass of cyaniding-3-glucoside chloride, 24825 molars absorptive at 520 nm in pH 1 solution and DF is the dilution factor (16).

Determination of ascorbic acid (Vitamin. C).
Ascorbic acid was assayed by using an iodometric titration method described by (17). 10g of Potassium iodine diluted in 150 ml distilled water, 5ml of solution mixed with 25 ml of sample and 5 ml of (1M HCL) then titrated with potassium iodate (0.43 g dissolved in 1000ml distilled water) starch solution used as an indicator.

**Determination of mineral content**

About 0.5gm of ash powder was digested by nitric acid HNO$_3$ and hydrogen peroxide H$_2$O$_2$ (5:1 v/v), after digestion the volume was made up to 25 ml distilled water. the flame photometer and X-ray was used for detection mineral content (18).

**RESULTS AND DISCUSSION**

**Moisture content, total soluble solids and density**

Physiochemical parameters are important for understanding quality and stability, in this study some physiochemical properties were showed in (Table 1). Moisture is a significant parameter that affects food processing, preservation and nutritional value. It also has associations with material balance, packaging, shelf life and food adulteration. In grapes, the moisture content is responsible for some physical, geometrical and density properties (19). High moisture content improves flavour but products with high moisture content have decreased shelf life (20). In this study, the moisture content of unripe black grape berries was found to be 74.6 %, higher than some of the fruits like avocado and banana at 62.3 and 71.8%, respectively, as reported by (21) also close to that of lime fruit at 75.6% (22). Total soluble solids (°Brix) is used to determine sugar concentration which indicates the fruit maturation. The Brix of unripe grape juice was determined as °5.0 which is in range 3.3–9.9 that reported by (3), at 4.5–7.5 (4) and at 3–8 and 3.5–8 (2). The density was found to be similar verjuice from France and Germany, at 1.03 (1).

**Titratable acidity and pH**

Titratable acidity and pH can be used as a standard criterion to evaluate the maturation and quality properties of fruit juice. Total titratable acidity is expressed as tartaric acid with respect to the abundant acid in all grapes (23,24). In this study, total titratable acidity was determined as 30 g/l (3%), which is similar to that found for yediveren, Müşküle unripe grapes as well as other types reported by (4) and (2), 30g/l and 3% respectively. Further, this result corresponds is in line with (3) at 17.4–38.8 g/l and (1) at 24.8–30.0 g/l. In this study, a pH of 2.35 was found for verjuice, which is similar to the results found by Kalacik karasi and Müşküle unripe grapes at 2.35 and 2.37, respectively (2).

**Ash and crud fiber**

Ash in food represents the inorganic residues remaining after an organic matter is burned. It is regarded as a general measure of quality, with crud fibre being an organic residue. In this study, the ash content of verjuice 0.47 % of verjuice was found to be higher than some types of white unripe grapes at 0.33–0.41 (4), and 0.07 (25), and the crude fibre value was 4.5%, indicating as a good source of dietary fibre; the British Nutrition Foundation recommended daily intake of fibre is 17.2–20.1g/day for women and men, respectively.

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Vitamin C (ascorbic acid)

Vitamin C (ascorbic acid) is an important vitamin have therapeutic and antioxidant properties. More than 90% of vitamin C in the diet is supplied by fruits and vegetables including potatoes. It is also a natural antioxidant and facilitates the absorption of inorganic iron and reduces cholesterol \(^{(26)}\). Unripe fruits contain a high ratio of vitamin C compared to ripe fruits \(^{(27)}\). In the present study, vitamin C was found at a rate of 16mg/100ml, which is higher than that reported by \(^{(25)}\) for verjuice 12.25mg/100ml. comparing verjuices with other ripe fruits as for example 6.46, 14 and 9 mg/100gm for apple, mango and pineapple, respectively \(^{(17)}\).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Verjuice sample (Means+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>74.65±1.7</td>
</tr>
<tr>
<td>Total titratable acidity %</td>
<td>3.01±1</td>
</tr>
<tr>
<td>Total soluble solid (°Brix)</td>
<td>5.03±0.00</td>
</tr>
<tr>
<td>Crude fibre %</td>
<td>4.50±0.14</td>
</tr>
<tr>
<td>Vitamin C mg/100ml</td>
<td>15.0±0.64</td>
</tr>
<tr>
<td>pH</td>
<td>2.35±0.00</td>
</tr>
<tr>
<td>Ash %</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>Juice content %</td>
<td>49.9±0.03</td>
</tr>
<tr>
<td>Density</td>
<td>1.02±0.00</td>
</tr>
<tr>
<td>Sugar-acid ratio</td>
<td>1.6±0.00</td>
</tr>
</tbody>
</table>

Organic acid profile

The organic acid content in unripe grapes is the main criteria for using directly or as a natural sour taste in other food processing beverages. Furthermore, it can be used as an antimicrobial, antioxidant and anti-browning agent. Tartaric and malic acid are prevalent organic acids in grapes comprising 90% of total organic acids \(^{(28,29)}\). In this study, organic acids in unripe black grapes (table 2) and (Fig.4) were determined by using HPLC. The concentration of tartaric and malic acid was 15.2 and 7.4 mg/ml, respectively, for tartaric acid the content in three types of white unripe grape was ranged from 5.51–11.78 mg/ml, and from 6.25-14.05 mg/ml in three types of red unripe grape, these results are lower compared to the results we recorded. However, in the case of malic acid, we recorded a lower value of when white and red unripe grapes, ranging from 10.90–28.62 and 17.64–30.38 mg/ml, respectively, by \(^{(3)}\). On the other hand \(^{(30)}\) analyzed two types of red unripe grapes – Barbera type (B1, B2) and Merlot type (M2, M1) – and found the concentration of tartaric acid 13.19–13.95and 11.11–11.0mg/ml, respectively. The malic acid concentration in Barbera (B2–B1) was 16.90–16.97 mg/ml and for Merlot M2–M1 was 17.20–17.32mg/ml. Compared with our study, the malic acid was higher but tartaric acid was lower. Differences in organic acid content are due to genetic, cultural practice, ecological factors, degree of maturity, growing region and year \(^{(29,31)}\), there is a difference in distribution and location of main organic acids in unripe grape berries when tartaric acid
accumulating in the first stage of berry development, particularly in peripheral flesh, in contrast, malic acid accumulates in flesh cells at the end of the first growth phase. (32) also reported that grape berry gathering malic acid more rapidly than tartaric acid; however, during grape ripening the warm conditions lead to reductions in malic acid (33). Overall, the unripe berry is a transitional stage between the first (nouaison, véraison) and end growth phases of ripening (harvesting) in grape berry. This process is a set of metabolic interactions and respiration requiring energy. Both (32,34) showed that malic acid is used as a source of energy due to reduction; However, tartaric acid remains constant after the véraison stage.

Table 2: Organic acid concentration (mg/ml) of verjuice

<table>
<thead>
<tr>
<th>Organic acids</th>
<th>Concentration (mg/ml)</th>
<th>Retention time (min)</th>
<th>Standard Division</th>
<th>Standard Error</th>
<th>Correlation coefficient r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tartaric acid</td>
<td>15.2</td>
<td>7.35</td>
<td>0.9</td>
<td>0.64</td>
<td>0.999</td>
</tr>
<tr>
<td>Malic acid</td>
<td>7.4</td>
<td>8.22</td>
<td>0.5</td>
<td>0.38</td>
<td>0.986</td>
</tr>
</tbody>
</table>

Figure 4: Chromatograms of tartaric and malic acid concentration in unripe black grape juice (verjuice)

Minerals content

In this study, some minerals were determined for unripe black grape juices. The result showed in (Table 3), potassium recorded a high percentage ratio % 0.085 comparison to other minerals. Despite that iron also presented an acceptable range of 0.39 mg/ml. the daily value recommendation depends on food and drug administration for adults are using 3500 mg potassium and 18mg iron because that unripe black grape juice can be used as a rich source of these minerals in the human diet. Unripe grape is the transition stage the composition of grapes are changed, (35) reported that the mineral concentration increase 2-4 folds during ripening. variety in mineral concentration back to the grape type, ability of absorption inorganic minerals,
temperature, soil, fertilizer, sunlight exposure and rainfall. As well as, variety in mineral composition has an effect on sensory properties of grape and its products (36).

Table 3: Minerals concentration in unripe black grape juices (verjuice)

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Calcium</th>
<th>Magnesium</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Iron</th>
<th>Zinc</th>
<th>Coppers</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>mg/ml</td>
<td>mg/ml</td>
<td>mg/ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.072</td>
<td>0.077</td>
<td>0.034</td>
<td>0.085</td>
<td>0.39</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Phytochemical properties

Total polyphenolic compounds

Grapes, in particular, dark-coloured grapes and their products, are rich in phenolic compounds, also one of the most important quality parameters of grapes is the total phenolic compounds, which are beneficial to health in terms of cardiovascular disease and several types of cancer (37). Phenols in grapes and wine comprise the third most common constituent after carbohydrates and organic acids (38). In this study, phytochemical properties were determined then showed in (Fig.5). the total phenolic content in verjuice was 307 mg/l, which showed higher values 270.21–227.48 mg/100ml, in comparison those reported by (4), and lower 473mg/l than the mean value mentioned by (2) as well as compared to those values of 315–1330mg/l reported by (1), in France, Germany and Iran verjuice samples. The low phenolic ratio may be related to the distribution of phenolic compounds biosynthesis; ripe grapes were found to have less than 10 % in pulp, 60–70% in seeds and 28–35% in skin (39) but in unripe grapes, all parts have a smaller surface area and volume compared to ripe grapes. Despite that sunlight exposure, availability of water, high temperature decreases the total phenolic compounds (40). Furthermore, total phenolic content varied with cultivar, soil composition, climate, geographic origin and fungal infection (41).

Total antioxidant capacity

Antioxidants are used as a food preservative to protect food damaged by free radicals. Which is using a high dose of verjuice (14 ml/kg/day) is useful in lowering LDL levels (7). In the present study, total antioxidant capacity (in terms of gallic acid) of unripe black grape verjuice was found to be 36μg/ml by the DPPH method, while (42) reported a level of 47.19–92.21% and (25) found 91.98%. (2). The used ferric reducing ability of plasma (FRAP) and Trolox – equivalent antioxidant capacity (TEAC) assays and found 0.010–0.231 and 0.035–0.085 μMol TE/ml. These differences are likely to be related to the variety of assay choice as well as climate differences, soil type, harvest time, storage and laboratory conditions.

Total anthocyanin content.

Anthocyanin is the main parameter important in both technological and nutritional cases. Recently, these components have received increasing interest as nutritional antioxidants. Anthocyanin is located in the skin.
within the cell, primarily in vacuoles; the concentration of anthocyanin varies with a variety’s maturity, seasonal condition, production area and viticulture practices\textsuperscript{(43)}. Total anthocyanin content in black unripe grape verjuice was found to be 23.43mg/ml. There is a lack of information on anthocyanin content and phytochemical properties in all types of unripe grapes, and to the best of our knowledge, description of total anthocyanin content has not been previously reported, considering that unripe grape has a green color absent in anthocyanin. To compare with other studies, and due to not having an estimation of total anthocyanin in unripe grapes, we compared total anthocyanin content with white and pink ripe grapes,\textsuperscript{(44)} determined anthocyanin concentration in three types of white grapes. Sauvignon Blanc, Riesling and Chardonnay as well as two types of pink grape. Gewurztraminer and Moscateller, and found that the highest amount was observed in Gewurztraminer at a level of 1094.90μg/kg, compared with our study, which found that our samples were 20,000 times less rich in terms of total anthocyanin content. The variability in anthocyanin content may be related to the presence of certain genes, particularly UDP-glucose, flavonoid 3-O-glucosyltransferase (UFGT), and bioactive processes such as a methoxylation and acylation\textsuperscript{(44,45)}.

![Figure 5: Phytochemical properties of unripe black grape juice (verjuice)](image)

**CONCLUSION**

The present study leads to a display of the essential properties of verjuice made by pressing unripe black grapes to food manufacturers and health nutrition consultants. that unripe black grape juice is a rich source of main compounds such as antioxidants, anthocyanin, organic acids, dietary fibres, and Vitamin C. Furthermore, supporting vineyard workers before burning grapes by sunstroke in warm regions, harvesting the unripe grape begins relegation of losses and will be using into other food processing production.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
FUNDING: Self-funding

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