Nutritional and total phenolic analyses of unfermented and fermented red grape (Vitis vinifera) beverages

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**ABSTRACT**
Grape juices—fresh, homemade fermented and commercial non-alcoholic were evaluated for their nutritional, Physico-chemical, phenolic and anthocyanin content. The grape juices obtained were clear, purple, and pH ranging between 3.3-3.5. The titrable acidity increased in the commercial grape juice (8.65%) whereas total solids (22.67%) was higher in the homemade fermented samples. The nutritional parameters such as carbohydrate, protein, dietary fibres etc. increased in the commercial sample except for vitamin C, which was higher in the fresh juice sample. The total phenolic content (2650 mg/lit) and total anthocyanin content (310 mg/lit) of commercial grape juice was higher compared to the homemade fermented sample. The increase in various parameters in the commercial sample could be attributed to manufacturing and processing techniques used during the production of grape juice.

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**INTRODUCTION**
Grape is an important and popular fruit commodity with high economic and agricultural value. Mainly cultivated for the production of wine, the grape is largely consumed as fruit and processed into raisin, wine or juice (Orak, 2007). The production of grape juice around the world is estimated 11-12 million hectolitres with the United States of America, Spain and Brazil being the highest producers and consumers (OIV, 2003). Grapes are a rich source of phenolic compounds, which not only contribute to the flavour and organoleptic characteristics of the processed product but also impart health benefits, particularly antioxidant activity (Burin et al., 2010). The phenolic compounds present in grape, grape juice and red wine are mainly the flavonoids (catechins, procyanidins), anthocyanins and flavanols. Additionally, certain non-flavanoid compounds such as hydroxybenzoic acids and hydroxycinnamic acids are also present. These compounds are thought to provide antioxidant activity essential to tackle oxidative damage due to free radicals (Palomino et al., 2000; Krikorian et al., 2012; Deng et al., 2011).

Clinical studies have shown that consumption of fruits and vegetables rich in polyphenols reduces the risk of cardiovascular diseases as well as cancer. Red wine is said to have beneficial health effects compared to other alcoholic beverages due to its high phenolic content while non-alcoholic grape juice has also found to reduce atherosclerosis, ageing, Parkinson's disease, cataract, cancer etc. (Dani et al., 2009; Park et al., 2009). Grape juice has also reported to beneficially influence cognitive and motor function (Krikorian et al., 2012). The flavonoids found in grape juice such as catechin, epicatechin, anthocyanin, quercetin etc. are said to possess anti-inflammatory, antioxidant, platelet inhibitory activity as well as reduction of LDL oxidation and prevention of DNA damage as reported in in vitro and animal studies (Singletary et al., 2003; Xia et al., 2010).
The polyphenol concentration varies in different fruits and vegetables as well as in different grape juices. The concentration of polyphenols depends on the species/variety of grape, its age, ripeness, culture conditions and the processing technology used to obtain the juice (Dani et al., 2007; Natividade et al., 2013). Processing techniques such as extraction, heat and enzymatic treatment significantly affect the concentrations of polyphenols. The polyphenols also differ in the tissues of grape and are mainly contained in the skin, seed and pulp wherein the pulp is rich in phenolic acids, and the skin is high on flavonoid content (flavanols, flavonols and anthocyanins) (Naczk and Shahidi, 2006). Therefore, the objective of this study was to evaluate the colour, total phenolic content and antioxidant activity of fresh, homemade fermented and commercial non-alcoholic grape juices.

MATERIALS AND METHODS

Sample Collection
The grape (Vitis vinifera L.) cultivar was used in this study. The selected grapes were procured from Pazyamthur market, Salem, Tamil Nadu, India. The selected grapes were washed in potable water to remove soil particles and dirt. The grapes were hand-picked from the clusters, and the stems were removed carefully to obtain good quality grapes.

Preparation of juice
The grape juices were prepared using an HSC juicer (Omega NC900HDC Juicer Extractor) and a vertical type LSM juicer (SJ200B, Hurom Co., Ltd., Korea), respectively, according to the manufacturer’s instructions. For juice preparation, the same amount of water (1:1, w/w) was added to wash grapes before grinding for 5 min using a blender. The grape flesh (GF) was prepared from washed grapes by removing skins and seeds by hand. Each sample was used immediately in experiments.

Preparation of homemade fermented juice
Black grapes procured from the local market was washed and crushed along with stems. Water was then added and allowed to boil for half an hour. After cooling the brix was adjusted to 23°C by adding the appropriate amount of concentrated sugar solution. KMS, i.e. potassium metabisulphate was added at 0.01% (w/v) and mixed well to prevent any contamination. Must was kept for overnight before inoculation. Dry yeast (250 mg/l) was added to one batch and activated yeast (1% w/v) kept at 27°C for 24 h was added to another batch of 10 litres. Both the jars were then kept for fermentation at room temperature for 8 days.

Commercial juice
The commercial juice was brought from the local market of Salem district, Tamilnadu, India.

Proximate Analysis
Physico chemical analysis was done, such as estimation of TSS, pH, titrable acidity, and colour. The pH of the periodic samples was determined using Digital pH meter. Total soluble solids (TSS) in the juice were determined using Erma hand refractometer of 0-32 °Brix. Titrable acidity was expressed as per cent acidity and analyzed. Moisture content, ash content, crude fibre, crude protein and fat content were all determined using the methods as described by AOAC (2010). Carbohydrates Total carbohydrate was calculated using the formula:

\[ \%C = 100 - (\%P + \%F + \%A + \%W + \%Fi) \]

Where; \%C = percentage carbohydrates
\%P = percentage of protein \%F = percentage of fat
\%A = percentage of Ash \%W = percentage water
\%Fi = Percentage of fibre

Vitamin C was determined using methods as described by AOAC (2010).

Extraction of total polyphenol
The flavonoids and total polyphenols compounds were extracted according to the methods reported with slight modifications. Ten gram of sample was accurately weighed, placed in a beaker and 100 mL of methanol-water (80:20, v/v) was added. The mixture was poured into test tubes which were then covered with aluminium foils and finally placed into a water bath for extraction at a temperature range of 50°C-80°C within 1-3 hours. The whole solution was filtered through Whatman filter paper No. 42 (125 mm) and the filtrate was transferred into the rotary evaporator at 60 °C where the filtrate was allowed to evaporate into dryness. Then, 0.05 g of the extract was dissolved in 10 ml of solvent.

Determination of total polyphenol contents
Total polyphenol contents of the fruit extracts was measured using a modified colourimetric Folin-Ciocalteu method, with slight modifications. Fruit extracts (0.5 mL) were placed in a test tube. Folin-Ciocalteu reagent (2.5 mL) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 2 mL of sodium carbonate (7.5 per cent). Absorbance at 765 nm was read after 30 min. Gallic acid was used as standard and data were expressed as mg gallic acid equivalents (GA)/100 g dw using a calibration curve (Koksal et al., 2011).
Table 1: Physicochemical attributes of grape juice samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh Juice</th>
<th>Homemade fermented</th>
<th>Commercial (non-alcoholic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>Purple</td>
<td>Purple</td>
<td>Purple</td>
</tr>
<tr>
<td>pH</td>
<td>3.38±0.02</td>
<td>3.34±0.02</td>
<td>3.56±0.02</td>
</tr>
<tr>
<td>Total Soluble solids (%)</td>
<td>14.85±0.03</td>
<td>25.65±0.02</td>
<td>21.82±0.04</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>7.10±0.15</td>
<td>7.22±0.10</td>
<td>8.65±0.03</td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>12.67±0.03</td>
<td>22.67±0.05</td>
<td>12.70±0.20</td>
</tr>
</tbody>
</table>

Table 2: Nutritional evaluation in grape juice samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh Juice</th>
<th>Homemade fermented</th>
<th>Commercial (non-alcoholic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein (%)</td>
<td>0.44±0.02</td>
<td>0.37±0.02</td>
<td>0.62±0.02</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>0.12±0.04</td>
<td>0.18±0.03</td>
<td>0.4±0.10</td>
</tr>
<tr>
<td>Total Ash (%)</td>
<td>0.31±0.03</td>
<td>0.22±0.04</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Dietary fibre (%)</td>
<td>0.19±0.04</td>
<td>0.16±0.02</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>16.36±0.02</td>
<td>14.82±0.02</td>
<td>17.63±0.05</td>
</tr>
<tr>
<td>Sugars (%)</td>
<td>15.62±0.03</td>
<td>13.84±0.40</td>
<td>12.98±0.19</td>
</tr>
<tr>
<td>Energy (KCal)</td>
<td>67.17±0.76</td>
<td>62±1.00</td>
<td>64±1.00</td>
</tr>
<tr>
<td>Potassium (mg/lit)</td>
<td>121.91±0.86</td>
<td>109.67±1.53</td>
<td>105.33±2.08</td>
</tr>
<tr>
<td>Vitamin C (mg/lit)</td>
<td>26.77±0.25</td>
<td>24.32±0.28</td>
<td>20.25±0.28</td>
</tr>
</tbody>
</table>

Table 3: Total Phenolic content, total anthocyanin and antioxidant activity

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic compounds (mg/lit)</th>
<th>Total anthocyanin (mg/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Juice</td>
<td>2640</td>
<td>220</td>
</tr>
<tr>
<td>Homemade fermented</td>
<td>2380</td>
<td>240</td>
</tr>
<tr>
<td>Commercial (non-alcoholic)</td>
<td>2650</td>
<td>310</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Physicochemical attributes

A systematic study of the physical properties of food is relatively new scientific discipline. Physical properties of food help to identify the relationship of product quality as well as their effect on processing behaviour of foods. Below the table represents the three various products Physicochemical attributes of grape juice samples (Table 1).

The physical attributes of grape juice samples such as colour, clarity and pH along with total soluble solids, titratable acidity and total solids were analysed. No significant difference was found in the physical appearance of the samples. The results are presented in Table 1. Total soluble solids were found to be higher in homemade fermented juice and commercial grape juice compared to fresh juice. The titratable acidity was significantly higher in the commercial sample, whereas total solids was higher in a homemade fermented sample.

According to the Brazilian legislation (Brasil, 2000), the minimum value for total acidity should be 0.41. Our results are in accordance with the legislation. Titratable acidity, along with Brix values indicates the sweet taste and acidity of the grape juice. The acidity of a commercial sample thus indicated that it is higher in acidity compared to homemade and fresh juice samples. Fructose and glucose formed 99% of the total sugar content at the maturation of the grapes and expressed as total soluble solids (Orak, 2007).

Nutritional parameters

Fruits are essential for sound health. Consumption of fruits and fruit incorporated food products has been increased because of their high content of bioactive compounds. The bioactive compounds...
helps to prevent chronic disease also. Below the table discussing the nutritional evaluation in grape juice samples.

Table 2 presents the nutritional parameters for the grape juice samples. Commercial non-alcoholic grape juice contained a high amount of carbohydrate, protein, fat, dietary fibre compared to fresh juice and homemade samples. However, the potassium and vitamin C levels were higher in the fresh juice samples.

Phenolic compounds are secondary metabolites produced by plant tissues and its production increases as a defense mechanism against phytopathogens. The grape juice produced when the skins containing phenols are heated produces a purple colour characteristic of a grape juice high in phenolics (Dani et al., 2007). Vitamin C is an important component in the plant that protects against reactive species produced during respiration and photosynthesis. It also plays a role in cell growth and acts as a cofactor for several enzymes involved in the synthesis of secondary metabolites etc. (Soares et al., 2004) (Dani et al., 2007). The slight reduction in vitamin C content in commercial grape juice could be as a result of manufacturing and processing effects. Additionally, factors such as grape variety, climate, soil, processing techniques etc. greatly influence the nutritional values of the grape juice product (Fuleki and Ricardo-Da-Silva, 2003). It was noted that grape juices produced at large scale show a high amount of carbohydrates etc. as seen in our study. This could also be attributed to the manufacturing processes, however further studies are required.

**Total phenolic and Total anthocyanin (TA) content**

Total phenolic and total anthocyanin content is given in Table 3. The total phenolic content reduced in the homemade sample, whereas the anthocyanin content was higher in the commercial sample.

Processing techniques such as high temperatures during extraction and pasteurization and different storage conditions affect the total phenolic and anthocyanin content (Frankel et al., 1998). Exposure to light may also cause variations in the anthocyanin content. Previous studies carried out in Italian wines made of different grape varieties showed anthocyanin content in the range of 102 to 105 mg/lit. (Burin et al., 2010) also reported TA content in the range of 42 to 460 mg/lit. The reduction in the total phenolic content in a homemade sample could be due to processing methods, variations in temperature treatment or a variety of grape cultivar used, its maturity etc.

**CONCLUSIONS**

This study aimed at evaluating the differences in the parameters of freshly prepared grape juice, homemade fermented and commercial grape juice. The total soluble solids and total solids were higher in the homemade and commercial samples indicative of the processes and manufacturing practices, which also increased the phenolic content, thus giving a purple colour to the juices. The increase in carbohydrate content and reduction in vitamin C along with variations in the phenolic and anthocyanin content in the commercial sample and homemade samples could be attributed to manufacturing, processing, heat treatments, type of grape variety, maturity etc.

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