Phytonutrient Composition, Antioxidant Activity and Acceptability of Baked Product Incorporated with Grape Seed Extract

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Abstract

Grape seeds are good sources of phytochemicals and possess antioxidant properties. This study was carried out to evaluate the antioxidant activity of baked products incorporated with grape seed extract. Measured quantity of aqueous extracts taken from 10, 20 and 30 gram of grape seed were used for the preparation of muffin. Phytonutrients in grape seed extract, grape seed extract incorporated muffin and standard muffin were analyzed. Further the antioxidant activity of the extract and the muffins were determined by 1,1 diphenyl 2, picryl hydrazyl (DPPH) method. The results showed that the high amount of phytonutrients is present in muffin incorporated with grape seed extract. The results also indicated that the 20g of grape seed extract muffin had the highest overall acceptability among the other two variations. Whereas the antioxidant activity was higher in grape seed incorporated muffin than the standard muffin. Hence the result of the study points out that grape seed extract added of fruit or fruit-based products due to their bioactive compounds that have been recognized. The mechanism of their protective effects is based on neutralization of the free radicals involved in oxidative damage, and this mechanism can thus be considered as preventive for cancer, cardiovascular and neurodegenerative diseases (carotenoids and flavonoids), cataracts and age-related macular degeneration (zeaxanthin, lutein, vitamins C and E, Zn) [4,5].

INTRODUCTION

Social concerns about healthy food have led to a profound change in our consumption patterns and to permanent innovation by the food industry to satisfy the consumers. The industry tends to diversify its production lines and develop new functional, appealing and easy-to-eat products to improve the consumers’ choices. The market for baked products in recent decade is different; due to the easy availability and varieties of products [1,2]. The food industry has a challenge to produce low cost, nutritive and convenient foods. The offer of functional foods has increased, since they have the potential to health security, due to the bioactive compounds that are present or added to traditional formulations [3]. In order to meet this it is necessary to study the sources of phytonutrients and antioxidant compounds that are available.

The beneficial effects are associated with regular consumption of fruit or fruit-based products due to their bioactive compounds that have been recognized. The mechanism of their protective effects is based on neutralization of the free radicals involved in oxidative damage, and this mechanism can thus be considered as preventive for cancer, cardiovascular and neurodegenerative diseases (carotenoids and flavonoids), cataracts and age-related macular degeneration (zeaxanthin, lutein, vitamins C and E, Zn) [4,5].

Fruits and vegetables are the major foods that contain these compounds with functional properties. Among the fruits grape is gaining momentum as a functional food ingredient globally.

Grape (Vitis vinifera) is one of the world’s largest fruit crops with an annual production of more than 60 million metric tons and 80 percent of the production is used in wine production [6]. Around 10 million tons of grape pomace are produced as byproducts of this industrial wine production and on a dry matter basis 38–52% of the pomace is composed of grape seeds [7].

Grape skins and seeds produced in large quantities by the winemaking industry are increasingly used to obtain
functional food ingredients [8]. Grape seed is a better source of antioxidant constituents than skin of grape/wine byproducts. Functional ingredients of grape seed include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric and polymeric proanthocyanidins and phenolic acids [9]. The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders [10].

There are studies indicating that cookies enriched with antioxidants from grape seeds extract taste good and have an antioxidant level about 10 times higher than a regular cookie [11]. Addition of grape seed extract to white bread resulted in increased antioxidant activity and decreased levels of the detrimental glycating end-product in bread [12]. The objective of this study was to assess the phytonutrient composition and antioxidant activity of grape seed extract incorporated muffin and compare its acceptability, phytonutrient with a standard muffin.

MATERIALS AND METHODS

Seed materials

Grape seeds were collected from the Gandhi gram food processing unit. The seed were collected and washed several times with water and blanched in saline water for 10 minutes and sun dried for a period of 3 days. The dried grape seeds were powdered and stored in air tight container for further analysis.

Methods

Preparation of grape seed extracts: Two extracts with methanol and aqueous solvents was taken using 10, 20 and 30gm of grape seed powder. 10, 20 and 30gm of grape seed powder was extracted with 30ml of solvents in a shaking incubator at 45°C for 2, 4 and 6 hours. The mixture was filtered through muslin cloth. The extract was utilized for further analysis. The extracts used for antioxidant activity and phytonutrient analysis. This was done in order to understand the time required for the maximum extraction of phytonutrients and highest antioxidant activity.

Evaluation of phytonutrient composition of grape seed extract

Grape seed extracts were subjected to qualitative analysis of phytonutrients namely flavonoid, steroid, saponin, tannin, anthocyanin, coumarin, phlorotannin and alkaloids using methods given by Harborne (1973) [13]. All the nine extracts taken from 10, 20 and 30gm of grape seed for 2,4 and 6 hours at 45°C were tested for antioxidant activity using 1,1 Diphenyl –β- picryl hydrazyl (DPPH) radical scavenging method [14]. Quantitative estimation of total polyphenol [15], total flavonoids [16] and tannin [17] were carried out using aqueous extract taken form 20 and 30gm of grape seed. Since the antioxidant activity was high in 20 and 30gm of grape seed extract.

Preparation of muffin

Three variations of the muffins were prepared incorporating 25ml of aqueous extract taken by incubating the grape seed powder for 6 hours. Three extracts were taken from 10g, 20g, and 30g of the grape seed powder.

A standard muffin without incorporation of the grape seed extract was prepared as control. The three variations muffins with 25ml of extract taken from 10g, 20g, 30g of grape seed were coded as A, B and C respectively.

A muffin batter recipe contained white flour(150g) granulated sugar(100g), baking powder (4.5g), salt(0.5g), milk(150ml), vanilla essence(0.5ml), egg(3 in no) and butter(20g) and grape seed extract(25ml). A standard muffin was prepared without the addition of grape seed extract. Based on results of preliminary trials muffin trials were conducted according to 3 .4 factorial design. Each formulation was prepared and measured in duplicate.

Sensory evaluation

For sensory analysis, five panelists from the Home science Department of Gandhigram Rural University, Gandhigram, India were selected. The order of presentation of samples to the panelists was randomized. Colour, appearance, texture, taste, odour and overall acceptability of samples were rated on 1–9 scale (1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither like nor dislike, 6 – like slightly, 7 – like moderately, 8 – like very much, 9 – like extremely). Consumer acceptability of the products was also carried out. Fifty college going students were selected to assess the overall acceptability of the developed muffin, the same hedonic scale was used.

Shelf life assessment of muffin incorporated with grape seed extract

The muffins were stored at room temperature for a period of 10 days. Total Bacterial count on the 0th, 5th, 7th and 10th day were done using serial dilution method [18].

Estimation of phytonutrient composition of the muffin incorporated with grape seed extract and standard muffin

The phytonutrients namely flavonoids, tannin, anthocyanin, coumarin, phlorotannin, phytosterols, steroids, saponin, and alkaloids [12] and of total polyphenol [15], total flavonoids [16] and tannin [17] and were analyzed. The estimation of proximate nutrients, phytonutrient, minerals and vitamins of the products were carried out to understand the amount nutrients present in grape seed extract incorporated muffin, and standard muffin.

Antioxidant activity of grape seed powder and grape seed muffin

Antioxidant activity grape seed incorporated muffins and standard muffin were estimated by 1, 1 Diphenyl –β- picryl hydrazyl (DPPH) radical scavenging method [14]. The DPPH radical scavenging capacity was analyzed using spectrophotometer.

Statistical analysis

Data were analyzed using the Statistical Analysis. ‘F’ test was used to determine significant differences between the means sensory evaluation of developed muffin incorporated with grape seed extract based on p<0.05.
RESULTS AND DISCUSSION

The preliminary screening of the methanol and aqueous extracts of grape seed powder revealed the presence of secondary metabolites such as flavonoids, alkaloids, saponin, phytosterols, tannin, anthocyanins and the results are presented in Table 1.

Antioxidant activity of the grape seed extract

The scavenging effect of methanolic and aqueous extracts of grape seed powder. The comparison of % inhibition concentration of different extracts with different quantity and time of extraction is presented below in Table 2. There was significant increase in percentage of Radical scavenging activity of methanolic and aqueous extract with increase in quantity of grape seed and time of extraction.

The highest antioxidant activity of the grape seed was obtained in 6 hours extraction in 30gm grape seed aqueous extract which was found to be higher than the other extracts. It is clear that 6hours extraction time showed the highest antioxidant activity among the three variations. In 6 hours duration of the aqueous extract antioxidant activity was found to be 92 per cent in extract taken from 30gm grape seed followed by 90 percent in grape seed extract taken from 20gm and 88percent in methanol extract taken from 30gm grape seed.

In a similar study it was reported that the extraction temperature of grape seed was optimum at 50ºC for 6hrs. In their study it was found that using 7.5 g of grape seed for 6 hours extraction was the most efficient condition. The increase in the temperature to 50ºC improved the extraction yield compared to room temperature. This increase may be attributed to the softening of the grape seed tissue under the higher temperature. The high temperature and long time also destroyed the covalent bonding between phenols and proteins and polysaccharides that caused easier contact between solvent and polyphenols to obtain the maximum yield with the highest antioxidant property [19].

Standardization and acceptability of the muffin incorporated with grape seed extract

Grape seed muffin was prepared using three different quantities of grape seed powder extract namely 10, 20 and 30g and the sensory evaluation was carried out to find the acceptability of the developed muffin.

Phytonutrient composition of grape seed extract

The results of the phytonutrient composition of the extract taken from 20 and 30g of grape seed powder is presented in Table 3.

The result shows that the tannin, flavonoids and total polyphenol content of the grape seed powder were 164mg, 75.13mg and 82mg respectively in aqueous extract taken from 30gm grape seed powder. The tannin, flavonoids and total polyphenols content of aqueous extract taken from 20gm grape seed powder was 160 mg, 67mg and 77.67 mg/GAE respectively. This indicates that as the quantity of seed increased the phytonutrient content also increased. Grape seed is a better source of antioxidant constituents. Functional ingredients of grape seed include several flavonoids, flavanols, procyanidins and polyphenols. The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders [20].

Mean sensory evaluation of grape seed extract muffins

The overall acceptability of grape seed extract taken from 10g, 20g and 30g of grape seed extract incorporated muffins is presented in table 4.

The above table-5 shows overall acceptability of the muffin incorporated with grape seed extract taken from 10g, 20g, and 30g of grape seed. It was noted that the overall acceptability of muffin incorporated with extract taken from 10gram of grape seed (A) was 7.7±0.286, muffin with extract taken from 20g of grape seed (B) was 8.9±0.322 and 7.3±0.691in muffin prepared with extract taken from 30g grape seed powder(C). Thus from the result it is clear that muffin prepared with extract taken from 20gm of the grape seed powder had the highest score.

The prepared muffins were served to 50 respondents and they were given a 9 point hedonic scale to assess the consumer acceptability and the results of the score obtained are presented in Table 5.

It was noted that the overall acceptability of 10g of grape seed extract muffin was 7.7±0.286, 20g of grape seed extract muffin was 8.9±0.322 and 30g grape seed extract muffin was 7.3±0.691. Statistical tool 'F' test was used and there were no mean difference found between the three variations of the developed muffins.

Overall results indicate that GSF as a partial substitute for all purpose flour (APF) in a baked good produces a product that is both high in poly phenols and acceptable in consumer evaluations [21].

A study carried out by incorporating grape seed in bread has reported that grape seed incorporation enhanced the flavor of the product rather than the texture and color of the product. On sensory evaluation of the control and grape seed incorporated bread the results showed higher preference units for flavor of the grape seed bread [22].

CONSUMER ACCEPTABILITY OF GRAPE SEED MUFFIN

The prepared muffins were served to 50 respondents and they were given a 9 point hedonic scale to assess the consumer...
Table 2: Antioxidant Activity of Grape Seed Extract.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>10gm</th>
<th>20gm</th>
<th>30gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hrs</td>
<td>4hrs</td>
<td>6 hrs</td>
</tr>
<tr>
<td>Methanol</td>
<td>54</td>
<td>62</td>
<td>77</td>
</tr>
<tr>
<td>Aqueous</td>
<td>54</td>
<td>68</td>
<td>87</td>
</tr>
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</table>

Table 3: Phytonutrient Composition of Grape Seed Extract.

<table>
<thead>
<tr>
<th>Phytonutrient</th>
<th>Aqueous extract taken from 20gm</th>
<th>Aqueous extract taken from 30gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin (mg)</td>
<td>160</td>
<td>164</td>
</tr>
<tr>
<td>Flavonoids (mg)</td>
<td>67</td>
<td>75.13</td>
</tr>
<tr>
<td>Total polyphenol (mg/GAE)</td>
<td>77.67</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 4: Mean Sensory Evaluation of Muffin Incorporated with Grape Seed Extract.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Grape seed muffin (A)</th>
<th>Grape seed muffin (B)</th>
<th>Grape seed muffin (C)</th>
<th>'F' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>8±0.547</td>
<td>9±0</td>
<td>6±0.707</td>
<td>47.25</td>
</tr>
<tr>
<td>Colour</td>
<td>8±0</td>
<td>8.8±0.447</td>
<td>8±1</td>
<td>91.0</td>
</tr>
<tr>
<td>Flavour</td>
<td>7.4±0.894</td>
<td>9±0</td>
<td>7.9±0.447</td>
<td>21.0</td>
</tr>
<tr>
<td>Texture</td>
<td>8±0</td>
<td>8.4±0.894</td>
<td>7.8±0.447</td>
<td>25.0</td>
</tr>
<tr>
<td>Taste</td>
<td>8±0</td>
<td>8.6±0.894</td>
<td>7.2±0.447</td>
<td>91.0</td>
</tr>
<tr>
<td>Consistency</td>
<td>8±0</td>
<td>9±0</td>
<td>7.6±0.547</td>
<td>37.2</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.7±0.286</td>
<td>8.8±0.322</td>
<td>7.3±0.691</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Table 5: Consumer Acceptability of the Grape Seed Extract Incorporated Muffins.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>10g(A)</th>
<th>20g(B)</th>
<th>30g(C)</th>
<th>'F' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>7.6±0.942</td>
<td>8.9±0.1</td>
<td>7.0±0.9</td>
<td>58.39594</td>
</tr>
<tr>
<td>Color</td>
<td>7.8±0.404</td>
<td>8.9±0.4</td>
<td>7.7±0.5</td>
<td>140.6662</td>
</tr>
<tr>
<td>Flavor</td>
<td>8.0±0.965</td>
<td>8.8±0.4</td>
<td>7.5±0.4</td>
<td>140.9081</td>
</tr>
<tr>
<td>Consistency</td>
<td>7.6±0.586</td>
<td>8.6±0.7</td>
<td>7.3±0.5</td>
<td>104.129</td>
</tr>
<tr>
<td>Taste</td>
<td>7.9±0.303</td>
<td>9±0</td>
<td>7.1±0.7</td>
<td>140.6662</td>
</tr>
<tr>
<td>Texture</td>
<td>7.7±0.418</td>
<td>8.9±0.1</td>
<td>7.3±0.7</td>
<td>186.1557</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.7±0.286</td>
<td>8.8±0.322</td>
<td>7.3±0.691</td>
<td>648.616</td>
</tr>
</tbody>
</table>

Table 6: Antioxidant Activity of Plain Muffin and Grape Seed Extract Incorporated with Muffin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain muffin</td>
<td>52.39±0.780</td>
</tr>
<tr>
<td>Grape seed extract incorporated muffin (B)</td>
<td>80.48±0.670</td>
</tr>
</tbody>
</table>

acceptability and the results of the score obtained are presented in Table 5.

The results of the consumer acceptability of grape seed extract muffin showed that the score were high for 20g of grape seed muffin than the other variations. Among these muffins 20g has got the highest mean acceptability. Thus from the sensory evaluation and the consumer acceptability it may be concluded that 20g grape seed muffin is accepted than the other variations.

Phytonutrient composition of the plain and grape seed extract incorporated muffins

Phytonutrient screening was done to understand the presence of phytonutrients. It is found that flavonoids, alkaloids, saponin, phytosterals, tannin, anthocyanins were present in grape seed extract incorporated muffin (B) whereas coumarin, phlorotannin were absent in the two extracts whereas in the plain muffin only alkaloids, saponins and phytosterol were present. Phytonutrients having antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes. A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. Flavonoids may have the ability to protect against free radical attack in both aqueous and lipid environments, thus providing an effective antioxidant defense in biological systems. Pharmacological properties of phytonutrients, especially positive
Antioxidant activity of plain and grape seed extract incorporated muffin

The natural antioxidant have the capacity to improve food quality, stability and can also act as nutraceuticals to terminate free radical chain reaction in biological systems and thus may provide additional health benefits to consumers. The intake of antioxidants such as polyphenols has been effective in the prevention of various diseases [24]. The antioxidant activities of grape seed extract incorporated muffin and plain muffin are presented in Table 6.

The DPPH assay was used to detect the antioxidant activity of the muffin incorporated with extract taken from 20gm muffin and plain muffin. The results indicate a highly positive DPPH radical scavenging activity for grape seed extract incorporated muffin (B) than the plain muffin.

The radical scavenging activity was 80.48 per cent for grape seed extract incorporated muffin and 52.39 per cent for the plain muffin.

Phenolic compound present in grape seed probably would have involved in their antiradical activity [25].

The studies have established that an antioxidant may successfully reduce the risk of chronic illness, diabetes, Alzheimer’s and cardiovascular disease [26].

CONCLUSION

It may be concluded from the study that grape seed has a good quantity of phytonutrients are present in high amount in grape seed powder. The overall acceptability of the muffin incorporated with extract taken from 20g grape seed was higher than the other variations. The antioxidant activity of the grape seed extract incorporated muffin was higher than the plain muffin. Thus the result points out that grape seed extracts may be utilized for the preservation of food products as well as for formulation of health supplements and nutraceuticals to protect the body from degenerative diseases.

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