MORPHOMETRIC CHARACTERISTIC, NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF SARARANGA SINUOSA HEMSYLES (PANDANACEAE) DURING RIPENING: A NATIVE BERRIES OF PAPUA, INDONESIA

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Abstract

Fruit of Sararanga sinuosa Hemsley is called as “Anggur Papua” or “grape Papua” by local people. S. sinuosa is endemic to Papua and its fruits are classified as berries. Morphometric characteristic (weight and diameter), nutritional values: namely proximate composition, soluble solids content, titratable acidity, pH, pectin, ascorbic acid and analysis of antioxidant activity (DPPH assay) of the fruits at three different ripening stages were determined. The weight and diameter of S. sinuosa fruit did not differ at different ripening stage viz. At greenish-white, orange-red and red fruit stages. The nutritional value of S. sinuosa fruit showed that proteins content, fat and pH remained unchanged as colour of fruit developed from greenish-white to orange-red and red. However, total carbohydrates, soluble solids content, ratio of soluble solids content: titratable acidity, pectin and ascorbic acid were increased significantly, despite the decrease of TA. The antioxidant activity determined by DPPH method and be avowed as IC50 showed that greenish-white fruit and red fruit had higher values than orange-red fruits. Fruits of S. sinuosa are alike berries from morphometric characteristic and nutritional value point of view. Based on soluble solids content (SSC), titratable acidity (TA), ratio of SSC/TA and pH, fruits of S.sinuosa can be processed into juice, jam and jelly. The greenish-white and red fruit ripening stages of S. sinuosa are considered to be potential source of natural antioxidant.

Key words: Sararanga sinuosa Hemsley, Nutritional value, DPPH method, Berries of Papua, Fruit colour changes.

Introduction

“Anggur Papua” (Sararanga sinuosa Hemsley) is one of the endemic species of Papua Island. The genus Sararanga belonging to the family Pandanaceae, has two species, viz. Sararanga philippinensis Merrill and Sararanga sinuosa Hemsley. Sararanga philippinensis species occurs at Philippines territory island, while Sararanga sinuosa Hemsley is confined to Papua, Papua New Guinea and other islands around it (The Solomon Islands and The Fauro Island) (Keim 2009; Purwanto & Munawaroh, 2010). S. sinuosa fruits are kidney-shaped, pale green when unripe and turns into red colour when fully ripened with soft outer skin (Stone et al., 1998; Keim, 2009).

Fruits of S. sinuosa are classified as berries and are usually consumed as berries. It has sweet taste when ripe and unripe fruit has plain taste (Lekitoo et al., 2012). Tribe Tepra in Depapare, Jayapura and the community in Yapen have been consuming fresh fruits (Purwanto & Munawaroh, 2010; Lekitoo et al., 2012). Each tree can produce 6 to 10 bunches, each bunch weighs in between 10-20 kg, with clusters of fruiting bunches having about hundred fruits (Fig. 1A) (Purwanto & Munawaroh, 2010). This fruit has a potential source of food, particularly as a table fruit or processed into beverages and other fruit-based products (Lekitoo et al., 2012).

The morphometric characteristics (size and weight) of the berries are effected by different cultivars, shape, skin colour and fruit maturity stage. The weight and size of berries vary from plant to plant (Villagra et al., 2014; Rolle et al., 2015) and are influenced by maturity stage, such as in raspberry (Rubus idaeus L.) (4.9-6.7 g) (Stavang et al., 2015); arrayan berry (Luma apiculata) (0.33-0.98 g; 0.75-1.29x0.53-1.25 mm) (Fuentes et al., 2016), mulberry (Morus alba L.) (5.8-33.9 g/10 fruit) (Lee & Hwang, 2017), shape and fruit colour, such as chilean berry (Gadillhera pumila) (pepper white: 223 mg, 6.8x2.5 mm; round white: 240.8 mg, 4.2x3.7 mm; red: 597.3 mg, 7.2x6.6 mm; pink: 456.7 mg, 5.6x5.5 mm) (Villagra et al., 2014) and cultivars, such as saskatoon berry (Amelanchier alnifolia Nutt.) (0.79-1.66 g) (Zatlyn et al., 2005).

The berries contain protein, carbohydrate (including sugar), fat (usually less than 1%), dietary fiber, 15% soluble solids of sugar, carotenoids, vitamins (rich in vitamin C) and minerals. Phenolic compounds of the berries mainly contain flavonoids (anthocyanin and flavonols), phenolic acids and tannins (Nile et al., 2014; Skrovankova et al., 2015). Berries have pleasant aromas and flavors are economically valuable owing to the antioxidant activity with the presence of bioactive compounds and are in great demand as a functional food source (Skrovankova et al., 2015). The nutritional value of berries was influenced by ripening maturity stage as reported by Wang et al. (2009); Krüger et al. (2011), Mikulic-Petkovsek et al. (2015), Horvitz et al. (2017) and Lee & Hwang (2017). The chemical changes also occurred during ripening. During ripening, berries become soft, darker alongwith chemical changes such as sugar accumulation, organic acid reduction and increase in anthocyanin levels (Mikulic-Petkovsek et al., 2015).
Several kinds of berries such as blueberries, raspberries (Speisky et al., 2012), blackberries, red raspberry (Jakobek et al., 2007; Krüger et al., 2011; Speisky et al., 2012), strawberry (Jakobek et al., 2007) or native berries, such as ararú berry (Luma apiculata) (Fuentes et al., 2016), calafate (Berberis sp.), murtilla (Ugni molinae Turcz.), maqui (Aristotelia chilensis Stuntz) (Ruiz et al., 2010; Speisky et al., 2012), chokeberry (Aronia melanocarpa) (Jakobek et al., 2007), have been used as an antioxidant. The antioxidant activity of berries is influenced by the concentration of polyphenols (individual or in total), and the maturity stage. Total polyphenols concentration of berries plays a key role as an antioxidant activity, such as highbush blueberry (Castrejón et al., 2008), chokeberry, elderberry, blackberry, raspberry, strawberry and black currant (Jakobek et al., 2007), mulberry (Lee & Hwang, 2017) and bog bilberry (Colak et al., 2016). According to various workers the anthocyanin played an important role in the antioxidant activity such as in raspberry (Wang et al., 2009), black currant, chokeberry, elderberry (Jakobek et al., 2007; Jakobek & Seruga, 2012), red raspberry, blackberry, strawberry, chokeberry, blueberry (Jakobek & Seruga, 2012). The ellagitannin and ellagic acid of berries also play a role in antioxidant activity, such as Andean blackberries (Hovitz et al., 2017). The maturity stage of berries also affect antioxidant activity, such as in mulberry (Lee & Hwang, 2017), red raspberry (Krüger et al., 2011) and arrayan berry (Fuentes et al., 2016).

No information is hitherto available on morphometric characteristic, nutritional value and antioxidant activity of S. sinousa fruit. Therefore, this is the first report on the morphometric characteristics, nutritional value and antioxidant activity of three different ripening stages of “Anggur Papua” fruit.
Material and Methods

Berry source: S. sinouosa fruit samples were collected during the period of July to September 2016 from three plants of wild population growing in forests around Bumi Perkemahan Pramuka Waena, Jayapura, Papua, Indonesia. The fruits of “Anggur Papua” were harvested from the whole bunch of grape and immediately transported by car to Laboratory of Biology, Faculty of Mathematics and Natural Science, Cenderawasih University for sample preparation.

Sample preparation: Samples were taken randomly from three fruiting bunches and separated according to the colour of the fruit ripening stage (greenish-white, orange-red and red) (Fig. 1B). For fruit morphometric determination, the colour of the fruit ripening stage was noted at Laboratory of Biology, Faculty of Mathematics and Natural Science, Cenderawasih University, Jayapura, Papua, Indonesia. The samples were stored in a polyethylene bag (18x23 cm) and placed in a styrofoam container (55x45x30 cm³) with blue ice for determination of nutritional value and antioxidant activity. Samples after harvesting were transported on the same day (8 hours journey) by plane to Surabaya. Samples were directly brought to Malang district by car, stored overnight in a refrigerator (Electrolux, Indonesia) and transferred to a Laboratory, Faculty of Agricultural Technology, Brawijaya University for further analysis.

Determination of physical characteristic: Fruit fresh weight based on fruit colour stage of ripening was individually measured by a digital balance (Adam, England). Fruit polar and equatorial diameter were also recorded by a digital vernier caliper (Mituyo, Japan). Seventy-five fruits from three different fruit maturity stages, namely: Greenish-White (S1 maturity stage), Orange-Red (S2 maturity stage) and Red (S3 maturity stage), were randomly selected from three bunches of fruit for the determination of morphometric characteristics.

Determination of nutritional value: Proximate analysis of protein, lipid, ash and crude fiber was carried out as described by The official methods of Association of Official Analytical Chemists (AOAC, 2011). Proximate analysis was done for each sample from S1, S2 and S3 maturity stage with three replicates. Moisture content was determined using thermogravimetry method and expressed as % (percent) (AOAC 930.04). Kjeldhal methods was used to determine the crude protein content (N X 6.25) (AOAC 920.152). Total fat content was determined using Soxhlet method and expressed as % fat in dry basis. A known weight of sample was extracted in petroleum ether (boiling point 40-60°C) using soxhlet (AOAC 948.22). Alkalinity and gravimetric method was used to determine the ash content in the samples and expressed as % dry basis (AOAC 940.26). Crude fiber was determined using digestion method. A known weight of fat-free sample was digested with refluxing in 1.25% sulfuric acid and 1.25% sodium hydroxide (AOAC 930.10). The difference method was used for determination of total carbohydrate content in the samples. The total percentages of moisture, fat, crude protein, ash and crude fiber was subtracted from 100%.

For determination of soluble solids content (SSC) of titrable acidity (TA) and pH, fruits were homogenized and filtered. Three replicates of all the readings were taken. TA was determined by titrating the sample (2-3 ml diluted juice into ca 20 ml neutralized H₂O) with 0.1M NaOH using 0.3 ml phenolphthalein and expressed as a percent acetic acid equivalent (grams of acetic acid in 100 gram sample) (AOAC 942.15, 2011). SSC was determined using a refractometer at room temperature and expressed as Brix (AOAC 932.12, 2011) and the pH of the juice was obtained with pH-meter (AOAC 981.12, 2011).

Pectin content in sample was determined using gravimetric method and expressed as a percent (Rangana, 1978). Pectic substances are saponified with alkali and precipitated with calcium pectate by the addition of calcium chloride. The calcium pectate precipitate was washed until free from chloride and then dried and weighed.

Ascorbic acid was determined using 2,6-Dichloroindophenol Titrimetric Method (AOAC 967.21, 2011). Ascorbic acid was analyzed in triplicate for ripening stage. The fruit was homogenized and filtered. Aliquots of 100 ml prepared juice were added to equal volume of metaphosphoric acid-acetic acid solution. The 10 ml solution aliquot and blank was titrated with 2,6 Dichloroindophenol which was standardized to ascorbic acid standards. Ascorbic acid was determined using the following equation:

\[ \text{mg ascorbic acid/mL} = (X-B) \times (F/E) \times (V/Y) \]

where X = average ml for test solution titration, B = average ml for test blank titration, F = mg ascorbic acid equivalent to 1 ml indophenol standart solution, E = number of ml assayed, V = volume initial test solution and Y = volume test solution titrated.

DPHH radical scavenging assay: The antioxidant capacity was determined according to the method proposed by Jakobek et al., (2007), each sample (± 250 g) was processed in juice extractor (Juicer, Phillip) in order to obtain natural fruit juice. Three replicates of juice (10 ml each) were centrifuged at 4000 rpm for 1 h. All juices were analyzed on the same day when the samples were ready.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to the method of Brand-Williams et al., (1995) with slight modifications (Jakobek et al., 2007). In the DPPH method, five dilutions of each fruit juice with three replicates were analyzed. The solution was prepared by mixing 50 µL of diluted fruit juice with 300 µL of methanolic DPPH- solution (1 mM) and was brought to 3 mL with the addition of methanol than was kept in dark at room temperature for 15 minutes. The absorbance samples was read against the prepared blank (50 µL diluted fruit juice, 2950 µL methanol) at 517 nm. Blank solution of DPPH was prepared (300 µL of 1mM DPPH solution, 2.7 mL of methanol) and was measured daily. The reduction of the DPPH radical was determined by the OD measurement at 517 nm in a UV/Vis spectrophotometer and the results were expressed as IC₂₀ (mg/mL).
Statistical analysis

Data was subjected to analysis of variance (ANOVA) and the Tukey test to identify significant differences between the three colour stages of ripening. Mean values with p<0.05 were considered statistically significant.

Result and Discussion

Morphometric characteristic: Biophysical observation of S. sinuosa fruit was carried out to get a general picture of fruit bunches including variability in length and weight of fruit bunches, individual fruits, seeds and the colour of fruits at different stages of ripening. Our results were consistent and showed variation in the morphometric characters of fruits, seeds and colour stage of fruit ripening. Fruit bunches of S. sinuosa are shown in Fig. 1A. The average of berry length and weight was 1.38 ± 0.01 m and 16.02±0.88 Kg, respectively (Table 1). Three different ripening stages of S. sinuosa are shown in Fig. 1B, 1, 2, 3. Fruits with more green colour were considered as unripe, whilst greenish-white were also taken as ripe fruit. Local people of Papua consumes orange-red and red colour fruits. Fig. 1 C, D and E exhibits variability in the individual seeds. Morphometric analysis of Armenian grape cultivars also showed wide variation in size, weight, colour and shape of the berries (Aroutiounian, 2015).

Table I. Bunches physical characteristic of S. sinuosa H. Fruit.

<table>
<thead>
<tr>
<th>Bunches character</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (m)</td>
<td>1.38 ± 0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>16.02±0.88</td>
</tr>
</tbody>
</table>

There were no significant changes in the average individual fruits weight (g), polar and equatorial diameter (mm) among white-green, orange-red and red colour fruit ripening stage (Table 2). Generally, a fruit will reach the maximum size before ripening (Anon., 2010). Ripening is the whole of all the changes that occur after the fruit reaches maximum size and before the fruit is damaged (Fry, 2017). The change of colour is one of the most clear and interesting characteristic associated with ripening, aside from to changes in aroma, taste and texture (Bouzyen et al., 2010; Fry, 2017). The growth of berry exhibits a double-sigmoid curve with three distinct stages (I, II, III) of berry development that is change in weight, volume and diameter. The increase in size and weight of berries occur in stages I and II, while stage III is the ripening stage of the fruit having maximum growth (Anon., 2010; Anon., 2010). Diameter of berries affect the physical properties and chemical composition. Therefore, this parameter can be used to determine the quality of berries, which could influence the consumer acceptance of ‘ready-to-eat’ fruit (Rolle et al., 2015).

Nutritional value: Proximate analysis of "Anggur Papua" at three different ripening stages is given in Table 3. It was noted that moisture, ash content and crude fiber of greenish-white fruit at ripening stage were decreased significantly at p=0.05, when the fruit developed orange-red and red colour at ripening stage. Total carbohydrate by difference was increased significantly from 10.72± 0.11% to 11.97± 0.05% as the fruit maturity stage developed to further ripening stage. Aubert & Chalot (2018) determined total sugar content by HPLC method in six France table grapes, exhibiting the variation from 3.97 to 5.63%. Berries are generally rich in water content between 75.5-93.25% (Hassimotto, 2008; Nile et al., 2014). In the present study crude protein and fat remained unchanged from greenish-white to orange-red and red at fruit ripening stage (Table 3). Berries contain protein and fat which are typically less than 1% and 15% of dietary fiber (Nile et al., 2014; Skrovankova et al., 2015). The composition of berries varies depending on cultivars, growing places and environmental conditions, nutrition, maturity, harvest time, and fruit storage conditions (Skrovankova et al., 2015).

SSC, TA, SSC/TA ratio, pH, Ascorbic acid, pectin and IC₅₀ of greenish-white, orang-red and red fruit S sinuosa are given in Table 4. SSC of red fruit ripening stage of “grape papua” was significantly higher (13.59%) than both greenish-white (12.56%) and orange-red fruit ripening stage (12.70%). Sugars at Scuppernong grapes began to accumulate at veraison and leveled at the ripening stage. Furthermore, at harvesting stage total sugars of Scuppernong grapes contained 9.45% (Johnson & Carrol, 1973). During the ripening stage there was an increase in SSC and a decrease in TA. Increase in SSC and decreasing of TA are also reported in highbush blueberry (Vaccinium corymbosum L.) (Castrejón et al., 2008), lowbush blueberry (Vaccinium angustifolium) (Gibson et al., 2013), grape (Vitis vinifera L. var Red Globe and var Crimson Seedless) (Segade et al., 2013), arrayan berry (Luma apiculata) (Fuentes et al., 2015), mulberry (Morus alba L.) (Lee & Hwang, 2017) and andean blackberries (Rubus glaucus B) (Horvitz et al., 2017). Ratio of SSC/TA of raspberry fruit was influenced by cultivar, storage conditions and ripening stage (Krüger et al., 2011).

Xie et al., (2009) reported that the acidity of berry grape was increased during the initially berry growth and after the lag phase acidity declined sharply. Our findings were in accordance with those of Xie et al., (2009) whereby the lowest TA of “grape papua” was at red fruit ripening stage. One of the main features of the ripening process in berries grape is accumulation of sugar and is of major consideration for the grape grower (Davies & Robinson, 1996). SSC/TA ratio is considered as the most realible parameter of fruit flavor (Parson & Paull, 2007). SSC/TA ratio of red fruit ripening stage was the highest and significantly different at p<0.05 (Table 3), therefore, the red stage of fruit ripening is considered to be the best time to consume “grape papua” fruit. Girardin & Sinha (2006) were of the opinion that standard SSC/TA ratio for fruit juice was 7.5 Brix, in relation to standard. The red stage of fruit ripening of S. sinuosa of “grape papua” is best for making juice.

S. sinuosa fruit at three colour stages of fruit ripening showed not significant difference (p<0.05) in pH. Similar data was reported in some other berries, such as Ribes species (Mikulic-Petkovsek et al., 2015) and strawberries (Kafkas et al., 2007). Fruit paste with pH at 2.8-3.4 is recommended for processing into a food product such as jam or jelly (Anon., 2016), emphasizing some pectin is present in fruit paste.
Ascorbic acid and pectin of *S. sinuosa* were increased significantly (*p*<0.05) at the red fruit ripening stage. Ascorbic acid in fruit increases as the fruit develops to ripening state, such as: from white-tip colour stage to red colour ripening stage of strawberry (Shin et al., 2015) and during ripening of arrayan berry (Fuentes et al., 2016). Vitamin C content is also affected by fruit ripeness and the loss of pectin and the depolymerization of cell wall and middle lamella modification (Brumwell, 2006; Goulao & Olivieria, 2008).

It has been also reported that SSC, TA and ratio of SSC/TA has also affected the density of berries (Segade et al., 2013). The density of berries also influences the physicochemical properties which lead to berries quality (Rolle et al., 2015). Therefore, chemical characteristic such as SSC, TA and sugar-acid balance of berries are important attributes for consumer acceptance (Segade et al., 2013). Nutrition value of berries information at different maturity stages varied at different harvesting time (Arif et al., 2010).

### Table 2. Morphometric characteristic of colour stage of ripening *S. sinuosa* H. Fruits

<table>
<thead>
<tr>
<th>Colour stage of ripening</th>
<th>Fresh weight (g)</th>
<th>Polar diameter (mm)</th>
<th>Equatorial diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (Greenish-White)</td>
<td>0.97 ± 0.04a</td>
<td>16.78 ± 0.06a</td>
<td>10.83 ± 0.73a</td>
</tr>
<tr>
<td>S2 (Orange-Red)</td>
<td>1.10 ± 0.03a</td>
<td>18.00 ± 0.91a</td>
<td>11.32 ± 0.73a</td>
</tr>
<tr>
<td>S3 (Red)</td>
<td>1.33 ± 0.03a</td>
<td>18.49 ± 0.92a</td>
<td>11.59 ± 0.54a</td>
</tr>
</tbody>
</table>

Results are mean of 75 fruits ± standard deviation. Value with the same letter within the same column are not significantly different by Tukey’s test at *p*<0.05

### Table 3. Proximate analysis (g/100 g fresh weight) of colour stage of ripening *S. sinuosa* H. Fruits

<table>
<thead>
<tr>
<th>Proximate</th>
<th>Colour Stage of Ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Greenish-White</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>0.56 ± 0.01a</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>0.49 ± 0.01a</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>87.44 ± 0.08a</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>0.72 ± 0.01a</td>
</tr>
<tr>
<td>Carbohydrate by different (%)</td>
<td>10.72 ± 0.11b</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>11.41 ± 0.02a</td>
</tr>
</tbody>
</table>

Result are mean of triplicate ± standard deviation. Value with different letter within the same line are significantly different by Tukey’s test at *p*<0.05

### Table 4. Chemical characteristic and radical scavenging activity of colour stage of ripening *S. sinuosa* fruits

<table>
<thead>
<tr>
<th>Chemical characteristic</th>
<th>Colour stage of ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Greenish-White</td>
</tr>
<tr>
<td>Soluble solid content (%)</td>
<td>12.56 ± 0.08b</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>2.58 ± 0.08a</td>
</tr>
<tr>
<td>Ratio of soluble solid content and titratable acidity</td>
<td>4.88 ± 0.11a</td>
</tr>
<tr>
<td>pH</td>
<td>3.86 ± 0.001a</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 gram fresh weight)</td>
<td>11.32 ± 0.4b</td>
</tr>
<tr>
<td>Pectin (%)</td>
<td>0.14 ± 0.001b</td>
</tr>
<tr>
<td>DPPH IC₅₀ (mg/ml fresh weight)</td>
<td>12.49 ± 0.35b</td>
</tr>
</tbody>
</table>

Result are mean of triplicate ± standard deviation. Value with different letter within the same line are significantly different by Tukey’s test at *p*<0.05

**Antioxidant activity:** The results of antioxidant activities of “grape papua” juice at three different ripening stages are shown in Table 4. The IC₅₀ for the free radical scavenging activity of “grape papua” juice prepared from stage I to stage III fruits were 12.49±0.35, 17.62±3.49 and 12.23±0.46 mg/ml, respectively. Thus “grape papua” juice has a stronger scavenging effect on free radical at the full ripening stage than at the other stages, despite an increase in the IC₅₀ value at the 2nd stage. Fully ripened fruit had the lowest IC₅₀ value (12.23±0.46 mg/ml), although it was not significantly different at *p*<0.05 from stage I. Similar results were also reported by Yang et al., (2016), where full ripened mulberry extract had the lowest IC₅₀ value (10.08±1.12 ug/ml) and in green and mature fruits of red raspberry (*Rubus ideaeus* L.) by Wang et al., (2009).
Conclusion

The most obvious appearance of fruiting bunches of Sararanga sinousa Hemsley was the size of fruit bunches which reached to the average value of 1.38±0.01 m. Morphometric characteristic studies, nutritional value and IC50 value of Sararanga sinousa Hemsley fruit revealed that the fruit of “grape papua” were considered to be berry in general. Morphometric studies at three different ripening stages exhibited no increase in size and weight at ripening stage I, II and III. Decrease in moisture content, crude ash and crude fiber was observed at ripening stage I, II and III, on the other hand, total protein and fats remained unchanged. Increase in SSC value, SSC/TA ratio, ascorbic acid value and pectin value were noted at three different ripening stages, whereas TA value was decreased. Chemical composition studies of SSC, TA, SSC/TA ratio, pH and pectin value revealed that fruit of S. sinousa could be used in the processing of juice, jam and jelly products. The ripening stage of fruits at greenish-white and red ripening stages showed could be a potential source of food antioxidant. Further detailed work is needed to unveil the existence of a bioactive compound in “grape papua” for food, medicine and other related-food industrial applications.

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