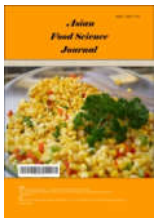

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Effect of Different Processing Conditions on Bioactive Compounds of Selected Grape Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Author MMWA conducted the research, conducted the statistical analysis and prepared the initial draft of the manuscript; Author RAUJM supervised the study and guided the data collection and revised the manuscript, Author ATA reviewed the manuscript, contributed to the preparation of the draft, and contributed to the presentation and analysis of data, Author GODS reviewed the manuscript and contributed to the interpretation and presentation of data, Author RM provided supervision, guided the study and reviewed data. All authors read and approved the final manuscript.

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ABSTRACT

Bioactive compounds in grapes vary in terms of cultivar and processing conditions. Raw juice and treated grape juices from locally grown Israel blue and locally available, imported, Red Globe and Michele Palieri varieties in Sri Lanka were used for the analysis. Grape juices were subjected to different processing conditions such as pasteurization and pectinase enzyme treatment. Total Monomeric Anthocyanin content (TAC), Total Phenolic Content (TPC), and antioxidant activity were analyzed. Compared to the imported grape varieties, the locally grown, pectinase enzyme-treated Israel blue grape juice with 2% pectinase enzyme concentration, 40 ^{0}C incubation temperature, and 2 hours incubation time, under dark condition had the significantly highest values ($p < 0.05$) for TAC at 177.03±4.15 mg/L of malvidin-3-O-glucoside (M3G), TPC at 527.07 ± 3.55 mg/L of Gallic acid equivalents and antioxidant activity in terms of DPPH radical scavenging assay with IC_{50} value at 7.05±0.35 mg/mL Gallic acid equivalents and ABTS radical scavenging assay

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with IC_{50} value at 0.31±0.01 mg/mL of Trolox equivalents. TAC, TPC, and antioxidant activity of three grape varieties showed the highest values in pectinase enzyme-treated grape juice which was followed by raw juice and the pasteurized juice respectively. This research has taken an approach to enhance the bioactivity of grape juices via pectinase enzyme treatment and evaluate the suitability of locally grown Israel blue grape variety in Sri Lanka to form as a functional beverage to meet nutritional and health requirements.

Keywords: ABTS, anthocyanin content; antioxidant activity; bioactivity; DPPH; israel-blue grapes; michele-palieri grapes; pasteurization; pectinase enzyme; red-globe grapes; total phenolic content.

1. INTRODUCTION

Consumption of grapes or grape-based products (i.e. red wine and grape juice) has been associated with various health benefits due to the high availability of polyphenols, which have proven to reduce oxidative stress and act as dietary therapeutics that could inhibit low-density lipoprotein (LDL) oxidation, attenuate the development of atherosclerosis and other cardiovascular diseases, diabetes, chronic obstructive pulmonary disease (COPD), cancer and asthma [1-2]. The research mainly focuses on three table grape varieties (*Vitis vinifera* L.) namely, Israel blue, Red Globe and Michele Palieri. Israel blue is locally grown in Sri Lanka whereas, Red Globe and Michele Palieri are locally available but imported grape varieties to Sri Lanka. Grapes grown in Sri Lanka are either table grapes or wine grape varieties; no cultivar is grown or identified specifically for manufacturing of juice and Israel blue is the seeded grape variety commonly cultivated by the farmers in Jaffna, Sri Lanka [3]. According to the Department of Agriculture, Sri Lanka, Israel blue grapes are dark blue or black in color with round to oval shape berries. According to the Department of Plant quarantine, Sri Lanka, Red Globe from the USA, and Michele Palieri from Italy are some of the highly imported grape varieties. Red globe grapes are red in color. It is a seeded spherical-shaped grape with less thick skin. Red Globe grape contains many vitamins (i.e. vitamin E, vitamin B6, vitamin B1); amino acids (i.e. arginine, glutamic acid, aspartic acid); and trace elements (i.e. iron, zinc, manganese) [4]. Michele Palieri is a Seeded grape with good vigor, fertility, and productivity. According to Carre et al. [5]. Michele Palieri grapes are blue-black in color with thick skin. Michele Palieri grape contains high anthocyanin content (Malvidin-3-O-glucoside as the main anthocyanin resulting in 660±60 mg kg^{-1} of fresh weight of grape berries) and therefore have a high level of suitability for the production of grape juice with nutraceutical properties [6].

Previous studies have described an inverse association between the consumption of fruits and vegetables and percentage mortality due to age-related diseases such as atherosclerosis and cardiovascular disease, Alzheimer's disease, diabetes, etc. [7]. This has been mainly due to the antioxidants, especially phenolic compounds in the diet [8]. Consumption of grape juice is associated with several health benefits in terms of increasing the antioxidant content, improving the endothelial function, inhibiting platelet aggregation, reducing plasma proteins oxidation, etc. [9-10]. The antioxidant properties of plant extracts are attributed to their polyphenol contents. Commodities containing a high level of phenolic compounds have great importance as natural antioxidants [11] and Grapes are among the fruits with the highest content of phenolic compounds [12]. Anthocyanin is a group of phenolic compounds found in grapes. Anthocyanins in grapes involve in many reactions that promote changes in the color of the juice, mainly through co-pigmentation and formation of polymeric pigments [13]. Color is one of the most important attributes used as a parameter to evaluate the grape juice quality. It is directly reliant on the phenolic composition of the juice and the anthocyanins present in the grape skin [14]. The quantity and composition of phenolic compounds and anthocyanins differ according to the species, variety, maturity of the grapes, weather, viticultural practices, and the region where the grapes are grown [15].

Different methods and treatments including the type of extraction, enzyme contact time with the pulp, the heat used throughout the production of grape juice have a significant impact on the final phenolic composition, compared to raw juice in natural form. Previous studies report that high temperatures used during the extraction (>60^0C); storage (>24^0C); and pasteurization are responsible for the degradation of anthocyanins and total phenolics which decrease the color and the antioxidant content of grape juice [16]. The

addition of the pectinase enzyme is considered a complex process as it results in several important alterations in the chemical composition of grape juice. Pectinase enzyme is a saponifying and depolymerizing enzyme that causes the degradation of plant cell walls [17] Various phenolic compounds, including some flavonoids, are located within or are tightly associated with cell wall material [18]. Therefore, the addition of the pectinase enzyme facilitates the release of various phenolic compounds in the juice which is a positive alteration of the chemical composition towards the increment of phenolic quantity and bioactive properties of the juice [19-20].

Beverages manufactured using specific fruit cultivars with higher antioxidant content and bioactive compounds of potential health benefits enable to expand the market for fruit-based products. In recent years, phenolic compounds have gained much attention due to their health benefits. Since grapes contain health-promoting phenolic compounds (i.e. resveratrol, epicatechin, catechin) which contribute to the prevention of various chronic diseases, it has become one of the main focuses in terms of functional foods [21]. The focus on healthy food is growing and functional foods play a major role [22]. This research study was an effort to discover the potentials for the introduction of a novel functional beverage using locally grown Israel blue grape variety and evaluate its position in terms of locally available imported Red Globe and Michele Palieri varieties.

2. MATERIALS AND METHODS

2.1 Plant Materials

Grapes were chosen in fresh, ripen stage without any visual damages, from the *Vitis vinifera* L. variety Israel blue, cultivated in Jaffna, in the Northern region of Sri Lanka and were purchased from a generous farmer for the preparation of grape juices. Michele Palieri and Red Globe grapes in the fresh, ripen stage without any visual damages were purchased from local supermarkets and the grape varieties were confirmed by the Department of Plant Quarantine, Sri Lanka.

2.2 Chemicals

Analytical grade 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) *(ABTS),* disodium monohydrogen orthophosphate, 1, 1-diphenyl-2 picrylhydrazyl *(DPPH),* Folin-Ciocalteu reagent, Gallic acid, methanol, monosodium dihydrogen orthophosphate, potassium chloride, potassium persulfate, sodium acetate, sodium carbonate, sodium chloride, 5,7,8-tetramethylchroman-2- carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Sigma-Aldrich chemicals PVT LTD. Sri Lanka). Pectinase enzyme under the brand name PEC600, with enzyme activity of 6,000,000 U/ml was purchased from Sunson Industry Group Co., LTD. China.

2.3 Sample Preparation

Grape juices from three grape cultivars, (i.e. Israel blue, Red Globe and Michele Palieri) were subjected to a Pectinase enzymatic treatment for clarification as shown in Table 1, based on a method described by Toaldo *et al.* [20] with some further modifications. A preliminary study was used to determine the best treatment combinations that deliver high-quality juices in terms of TSS/TA ratio and anthocyanin content. The 2 selected combinations were 2 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time, and 1.5 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time. Grape juices were prepared as raw juice, pasteurized juice, 1.5 % pectinase enzyme-treated juice, and 2 % pectinase enzyme-treated juice. Seeds of the grapes were initially removed. Raw juice was prepared by blending and converting grapes into pulp. The pulp was manually pressed using a clean muslin cloth bag and filtered through a Whatman™ 1001-090 Grade 1 qualitative filter paper with a diameter of 9 mm and a pore size of 11µm. Pasteurized grape juice was prepared by pasteurizing the filtered raw juice under 90 ^{0}C for 5 minutes. Grape juice samples were prepared in triplicates and duplicate measurements were taken from each replicate. Grape juice samples were stored at (-18) °C for subsequent analysis.

2.4 Total Monomeric Anthocyanin Content

The total monomeric anthocyanin content of the grape juice samples was determined using the pH-differential method described by Giusti and Wrolstad [23] with some modifications. A two-fold dilution series of grape juice samples were prepared from each sample. The appropriate dilution factor for each dilution was selected using pH 1.0 buffer that gave the absorbance

between 0.0050-2.000 U at 520 nm, which is the photometric linearity range in the UV-Vis Spectrophotometer (Model HACH- DR 6000). Grape juice samples from the dilution series were prepared in pairs in both pH 1 (Potassium chloride buffer) and pH 4.5 (Sodium acetate buffer) according to the previously determined dilution factors and the absorbance was measured at 520 and 700 nm using the UV-Vis Spectrophotometer. The absorbance value (A) of the diluted samples were calculated using the following equation:

$$A = (A_{520}\text{-}A_{700})_{pH1.0} - (A_{520}\text{-}A_{700})_{pH4.5}$$

Total Monomeric Anthocyanin Content (TAC) was calculated using the following equation:

$$\text{TAC} = \frac{\text{A} \times \text{MW} \times \text{DF} \times 1000}{\mathcal{E} x\, l}$$

For Israel Blue grapes and Michele Palieri grapes TAC was calculated as mg of malvidin-3-O-glucoside (M3G) per L of juice using a molar extinction coefficient ($\mathcal{E}$) 28,000 $Lcm^{-1}mol^{-1}$, molecular weight (MW) 493.3 $gmol^{-1}$ for M3G, path length (*l*) as 1 cm and appropriate the dilution factor (DF) [24]. For Red Globe grapes TAC was calculated as mg of cyanidin-3-O-glucoside (C3G) per L of juice using a molar extinction coefficient ($\mathcal{E}$) 26,900 $Lcm^{-1}mol^{-1}$, molecular weight (MW) 449.2 $gmol^{-1}$ for C3G, path length (*l*) as 1cm and appropriate the dilution factor (DF) [25].

2.5 Total Phenolic Content (TPC)

The total phenolic content of the grape juice samples was measured using Folin-Ciocalteu reagent assay according to the method described by Singleton et al. (1999) with some modifications [26]. The absorbance was read at 765 nm. Gallic acid was used as the standard phenolic compound with a concentration range of 1-6 ppm. TPC content was expressed as Gallic acid equivalents in mg/L of grape juice.

2.6 DPPH Radical Scavenging Activity

For the DPPH assay, the procedure followed the method of Brand-Williams et al. (1995) with some modifications [27]. The absorbance was read at 517 nm. The percentage inhibition of absorbance was calculated according to the following equation.

$$\% \text{ inhibition} = \left[\frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}}\right] \times 10$$

$\text{A}_{\text{control}}$ - Absorbance value of the DPPH solution of the control sample

A_{sample} - Absorbance value of the DPPH solution in the presence of grape juice sample

IC_{50} value was estimated from regression analysis using the software MINITAB® 17. Gallic acid was used as the standard antioxidant.

Table 1. Types of grape juices prepared by three different grape varieties

Grape variety	Type of juice
Israel Blue	Raw juice
	Pasteurized juice
	Enzymatically treated juice (*1.5% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time)*
	Enzymatically treated juice (*2.0% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time*)
Michele Palieri	Raw juice
	Pasteurized juice
	Enzymatically treated juice (*1.5% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time*)
	Enzymatically treated juice (*2.0% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time*)
Red Globe	Raw juice
	Pasteurized juice
	Enzymatically treated juice (*1.5% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time*)
	Enzymatically treated juice (*2.0% pectinase enzyme/ 40^{0}C incubation temperature/ 2 hours incubation time*)

2.7 ABTS radical Scavenging Activity

For the ABTS assay, the procedure followed the method of Arnao et al. [28] with some modifications. The stock solutions 7 mM ABTS and 2.45 mM potassium persulfate were mixed in equal amounts and allowed to stand in the dark under room temperature for 12-16 hours. The resultant $ABTS^{\bullet+}$ radical cation was diluted with PBS (Phosphate Buffered Saline), pH 7.4, to give an absorbance value of 0.70±0.02 at 734 nm using UV-Vis Spectrophotometer. The absorbance was read at 734 nm. The percentage inhibition of absorbance was calculated according to the following equation.

$$\% \text{ inhibition} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 10$$

$A_{control}$ - Absorbance value of the $ABTS^{\bullet+}$ solution of the control sample
A_{sample} - Absorbance value of the $ABTS^{\bullet+}$ solution in the presence of grape juice sample

IC_{50} value was estimated from regression analysis using the software MINITAB® 17. Trolox was used as the standard antioxidant.

3. RESULTS AND DISCUSSION

3.1 Total Monomeric Anthocyanin Content (TAC)

Pectinase enzyme degrades cell wall components. Anthocyanins are located mainly in the skin of the fruit [29] and pectinase enzyme treatment facilitates the release of anthocyanins by disrupting cell walls [30]. As shown in Table 2, there is a significant difference in values obtained for Total Monomeric Anthocyanin contents among different types of treatments under different grape varieties at ($p<0.05$) level. For Israel Blue grapes and Michele Palieri grapes, TAC was calculated as mg of malvidin-3-O-glucoside (M3G) per L of juice and for Red Globe grapes TAC was calculated as mg of cyanidin-3-O-glucoside (C3G) per L of juice. Pectinase enzyme treatment increases the release of anthocyanins. The obtained values for anthocyanin content in grape juice match with the values recorded in [12,30]. The highest TAC content in Israel Blue grape variety was recorded as 177.03 ± 4.15 mg of malvidin-3-O-glucoside (M3G) per L of juice in 2% pectinase treated grape juice and the lowest was recorded as a mean of 12.39 ± 0.93 mg of malvidin-3-O-glucoside (M3G) per L of juice in pasteurized juice. The highest TAC content in Red Globe grape variety was recorded as 45.24 ± 4.37 mg of cyanidin-3-O-glucoside (C3G) per L of juice in 2% pectinase treated grape juice and the lowest was recorded as a mean of 2.25 ± 0.19 mg of cyanidin-3-O-glucoside (C3G) per L of juice in pasteurized juice. The highest TAC content in Michele Palieri grape variety was recorded as a mean of 144.58 ± 5.33 mg of malvidin-3-O-glucoside (M3G) per L of juice in 2% pectinase treated grape juice and the lowest was recorded as a mean of 19.80 ± 0.53 mg of malvidin-3-O-glucoside (M3G) per L of juice in pasteurized juice. The results confirm the positive significant effect of the enzymatic treatment on the increment of total anthocyanin content in grapes and the negative effect generated by heat treatment [31-32].

3.2 Total Phenolic Content (TPC)

Cell wall degrading enzymes such as the pectinase enzyme can improve the extraction of phenolic compounds from fruit skins. The enzyme-assisted release of phenolic compounds from the cell wall matrix occurs via enzyme-catalyzed hydrolytic degradation of the cell wall polysaccharides that are presumed to retain the phenolic compounds in the polysaccharide-lignin network by hydrogen or hydrophobic bonding [33]. According to (Meyer et al. 1998) a mechanism has been identified as the direct enzyme-catalyzed breakage of the ether and/or ester linkages between the phenolic compounds and the plant cell wall polymers [34]. As shown in Table 3, there is a significant difference among the Total Phenolic Content of different grape juices in different grape varieties ($p < 0.05$). The ascending order of TPC is recorded as pasteurized juice, raw juice, pectinase 1.5% treated juice, and pectinase 2% treated juice. The highest TPC content in Israel Blue grape variety was recorded as 527.07 ± 3.55 mg Gallic acid equivalents per L of juice in 2% pectinase treated grape juice and the lowest was recorded as 329.73 ± 9.91 mg Gallic acid equivalents per L of juice in pasteurized juice. The highest TPC content in the Red Globe grape variety was recorded as 350.63 ± 1.73 mg Gallic acid equivalents per L of juice in 2% pectinase treated grape juice, and the lowest was recorded as 115.46 ± 2.15 mg Gallic acid equivalents per L of juice in pasteurized juice. The highest TPC content in Michele Palieri grape variety was recorded as 248.25 ± 8.22 mg Gallic acid equivalents per L of juice in 2% pectinase treated grape juice and the lowest was recorded as a

mean of 88.52 ± 5.37 mg Gallic acid equivalents per L of juice in pasteurized juice. With regards to the TPC content of grape juices from different varieties, the highest TPC content was recorded in 2% pectinase treated Israel Blue grape juice, and the lowest was recorded in pasteurized Michele Palieri grape juice. According to Landbo et al. (2007) increase in enzyme concentration increases the yield of Total Phenolic content [35]. In addition, there is a decrease in phenolic content due to thermal treatment, and this statement match with the low phenolic contents recorded in pasteurized juice [36]. The results obtained for the TPC content of grape juices match with the values recorded in Lutz. et al. [25].

3.3 Antioxidant Content

Enzymes are able to degrade or disrupt cell walls and membranes, thus enabling better release and more efficient extraction of antioxidants [37]. The antioxidant content is determined in terms of the DPPH radical scavenging assay and ABTS radical scavenging assay.

3.4 DPPH Radical Scavenging Assay

As shown in Table 4, there is a significant difference among the IC_{50} values obtained for different grape juices under different grape varieties. The ascending order of the IC_{50} value indicates the descending order of antioxidant capacity. The ascending order of IC_{50} value from the lowest value to the highest value in all grape varieties is; 2% pectinase treated grape juice. 1.5% pectinase treated grape juice, raw juice, and pasteurized juice. Therefore the highest antioxidant activity was recorded in 2% pectinase treated juice, where the IC_{50} is the lowest and the lowest antioxidant activity was recorded in pasteurized juice, where the IC_{50} value

Table 2. Total Monomeric Anthocyanin content of grape juices under different varieties

Type of grape juice	Israel Blue grapes (mg/L)	Red Globe grapes (mg/L)	Michele Palieri grapes (mg/L)
Raw juice	30.60 ± 0.97^{c}	8.67 ± 0.30^{c}	28.83 ± 0.80^{c}
Pasteurized juice	12.39 ± 0.93^{d}	2.25 ± 0.19^{d}	19.80 ± 0.53^{d}
Pectinase 1.5% treated juice	125.10 ± 4.01^{b}	24.37 ± 0.79^{b}	110.41 ± 3.66^{b}
Pectinase 2% treated juice	177.03 ± 4.15^{a}	45.24 ± 4.37^{a}	144.58 ± 5.33^{a}

**Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c,d letters in same column are significantly different at (p < 0.05) level*

Table 3. Total Phenolic Content (Gallic acid equivalents in mg/L) of different grape varieties under different treatments

Type of grape juice	Israel Blue	Red Globe	Michele Palieri
Raw juice	368.78 ± 10.89^{c}	176.22 ± 1.93^{c}	137.06 ± 2.92^{c}
Pasteurized juice	329.73 ± 9.91^{d}	115.46 ± 2.15^{d}	88.52 ± 5.37^{d}
Pectinase 1.5% treated juice	412.21 ± 2.91^{b}	219.07 ± 2.8^{b}	166.12 ± 5.12^{b}
Pectinase 2% treated juice	527.07 ± 3.55^{a}	350.63 ± 1.73^{a}	248.25 ± 8.22^{a}

**Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c,d letters in same column are significantly different at (p < 0.05) level*

Table 4. DPPH radical scavenging assay based on IC_{50} value (mg/mL) in different grape varieties under different treatments

Grape variety	Raw juice	Pasteurized juice	1.5% pectinase treated juice	2% pectinase treated juice	Standard antioxidant
Israel Blue	13.53 ± 0.31^{b}	15.50 ± 0.27^{a}	12.00 ± 0.15^{c}	7.05 ± 0.35^{d}	0.0036 ± 0.00
Red Globe	67.87 ± 0.97^{b}	95.88 ± 1.84^{a}	19.08 ± 0.56^{c}	9.93 ± 0.25^{d}	
Michele Palieri	176.74 ± 3.47^{b}	279.74 ± 1.48^{a}	21.65 ± 1.30^{c}	11.08 ± 0.41^{d}	

**Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D (n=6). a,b,c,d letters in same row are significantly different at (p < 0.05) level*

Table 5. Results of ABTS radical scavenging assay based on IC_{50} value (mg/mL) in different grape varieties under different treatments

Grape variety	Raw juice	Pasteurized juice	1.5% pectinase treated juice	2% pectinase treated juice	Standard antioxidant (Trolox)
Israel Blue	8.49 ± 0.15^{b}	9.25 ± 0.12^{a}	1.36 ± 0.11^{c}	0.31 ± 0.01^{d}	0.041±0.000
Red Globe	45.12 ± 0.31^{b}	56.04 ± 1.83^{a}	16.13 ± 0.74^{c}	6.18 ± 0.09^{d}	
Michele Palieri	66.79 ± 0.70^{b}	77.01 ± 1.03^{a}	10.25 ± 0.17^{c}	6.36 ± 0.12^{d}	

**Data presented as mean values for triplicates with duplicate measurements in each replicate ± S.D*

is the highest. The lowest IC_{50} value in Israel blue grape variety was recorded as 7.05 ± 0.35 mg of Gallic acid per ml in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 15.50 ± 0.27 mg of Gallic acid per ml in pasteurized juice. The lowest IC_{50} value in the Red Globe grape variety was recorded as 9.93 ± 0.25 mg of Gallic acid per ml in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 95.88 ± 1.84 mg of Gallic acid per ml in pasteurized juice. The lowest IC_{50} value in Michele Palieri grape variety was recorded as 11.08 ± 0.41 mg of Gallic acid per ml in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 279.74 ± 1.48mg of Gallic acid per ml in pasteurized juice. Among all the grape varieties, the least IC_{50} value was recorded in 2 % pectinase treated Israel Blue grape juice which has the highest antioxidant activity and the highest IC_{50} value was recorded in Michele Palieri pasteurized juice which has the least antioxidant activity. The IC_{50} value of the Gallic acid standard was recorded as 0.0036 mg/mL. According to Chipurura et al. [38], thermal treatment reduces the antioxidant activity and it matches with the low antioxidant capacity or high IC_{50} values obtained for pasteurized juices in the experiment. According to Toaldo et al. [20], an increase in pectinase enzyme concentration contributes to higher antioxidant activity and it matches with the results obtained for pectinase treated juices with low IC_{50} values.

3.5 ABTS Radical Scavenging Assay

The ascending order of IC_{50} value from lowest value to the highest value in all grape varieties are; 2% pectinase treated grape juice. 1.5% pectinase treated grape juice, raw juice, and pasteurized juice (Table 5). Therefore the highest antioxidant activity was recorded in 2% pectinase treated juice and the lowest was recorded in pasteurized juice. The lowest IC_{50} value in Israel blue grape variety was recorded as 0.31 ± 0.01 mg of Trolox equivalents per ml of juice in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 9.25 ± 0.12 mg of Trolox equivalents per ml of juice in pasteurized juice. The lowest IC_{50} value in the Red Globe grape variety was recorded as 6.18 ± 0.09 mg of Trolox equivalents per ml of juice in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 56.04 ± 1.83 mg of Trolox equivalents per ml of juice in pasteurized juice. The lowest IC_{50} value in Michele Palieri grape variety was recorded as a mean of 6.36 ± 0.12 mg of Trolox equivalents per ml of juice in 2% pectinase treated grape juice and the highest IC_{50} value was recorded as 77.01 ± 1.03 mg of Trolox equivalents per ml of juice in pasteurized juice. When considering all grape varieties, the least IC_{50} value was recorded in 2% pectinase treated Israel Blue grape juice which has the highest antioxidant activity and the highest IC_{50} value was recorded in Michele Palieri pasteurized juice which has the least antioxidant activity. The IC_{50} value of the Trolox standard was recorded as 0.041 mg/ml. Phenolic compounds are the most abundant antioxidants in fruits and plant-derived beverages [39]. The results confirm the increment of the antioxidant activity of grape juice by the enzymatic treatment due to the release of phenolic compounds [40,41].

4. CONCLUSION

Results obtained in this study showed that grape cultivar, as well as the processing conditions, could make a significant impact on the anthocyanin content and phenolic content of grapes which can directly affect the color and the antioxidant capacity of grape juices. The Total Phenolic Content, Total monomeric Anthocyanin Content, and antioxidant activity contrasted significantly among the locally grown Israel blue grape cultivar and locally available, imported Red Globe and Michele Palieri grape cultivar. Since the highest phenolic and phytochemical properties were shown in pectinase enzyme-

treated Israel blue grapes, it can be concluded that locally grown grapes can be used to fulfill the nutritional and health requirements of the Sri Lankans in the form of a functional beverage.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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