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MMW Aponso
Department of Food Science and
Technology, Faculty of Applied
Sciences, University of Sri
Jaywardenepura, Sri Lanka

RAUJ Marapana
Department of Food Science and
Technology, Faculty of Applied
Sciences, University of Sri
Jaywardenepura, Sri Lanka

R Manawaduge
Cargills Agri Foods LTD,
Deamanhandiya, Sri Lanka

Physicochemical analysis of grape juice from Israel blue (*Vitis vinifera* L.) grape cultivar under different processing conditions and a comparison with Red Globe and Michele Palieri grape varieties

MMW Aponso, RAUJ Marapana and R Manawaduge

Abstract

Physicochemical properties of Israel blue grapes (*Vitis vinifera* L.) has been hardly addressed in literature despite its wide cultivation in Jaffna, Sri Lanka. This work evaluates the physicochemical properties of Israel blue grape juice compared with two imported grape varieties in Sri Lanka (Red Globe and Michele Palieri). Total Soluble Solids (TSS), Titratable Acidity (TA), pH, color and clarity of each grape cultivar subjected to four different processing conditions namely raw juice, pasteurized juice, 1.5 % pectinase enzyme treated juice with 40 °C incubation temperature, 2 hours' incubation time and 2 % pectinase enzyme treated juice with 40 °C incubation temperature, 2 hours' incubation time, were studied. Israel blue grape juice with 2 % pectinase enzyme treatment, 40 °C incubation temperature, 2 hours' incubation time resulted in 18.75±0.27 TSS, 0.72±0.01% TA, 3.61±0.012% pH, 0.039±0.003 absorbance in terms of clarity and 21.79±0.86° hue angle indicating red color.

Keywords: Israel blue grapes - Michele Palieri grapes - Red Globe grapes - Pectinase -Physicochemical

1. Introduction

Grapes belong to the family Vitaceae and genus *Vitis* [1]. Grape (*Vitis vinifera*) is one of the world's largest fruit crops. Grape is also one of the most commonly consumed fruits in the world both as fresh fruit (table grape) and processed fruit (wine, grape juice, molasses, and raisins) [2, 3]. Referring to the contemporary crop recommendations by the Department of Agriculture, Sri Lanka, there are mainly five local grape varieties, namely, Israel Blue, Cardinal, Black Muscat, which are table varieties; Muscat MI, which can be used as both table and wine variety and French MI which is a wine variety [4]. Grapes grown in Sri Lanka are either table grape or wine grape cultivars; no cultivar is grown or identified specifically for manufacturing of juice. Israel blue is the seeded grape variety commonly cultivated by Jaffna farmers [5]. The fruits of Israel Blue grapes are dark blue or black in color with a round to oval fruit shape and cylindrical bunch shape. Red Globe and Michele Palieri are imported grape varieties in Sri Lanka which are locally available in the market.

The use of enzymes in juices contribute to the increment of the juice yield. Pectinolytic enzymes are one of the important groups of enzymes used in fruit processing industry and are one of the upcoming enzymes of fruit processing industries. The pectic substances in fruits are constituents of cell walls and of intercellular layers of all higher plants, along with cellulose and hemicelluloses. These substances are also found in juices and saps and contribute to the texture of fruits. Pectins have a linear α -1, 4 linked chain of pyranosyl D-galacturonic acid molecules, which is referred to as the polygalacturonan, or galacturonan backbone. Grape pectins are 44-65% esterified [6]. Pectinases break down complex polysaccharides of plant tissues into simpler molecules and increase the juice quality [7].

The sensory characteristics of grape juice are mostly reliant on the balance among the sugars, acids, natural flavors, phenolic compounds and color components [8]. Consumers are not keen to compromise either sensory quality or convenience when opting for health benefits in food products [9]. Hence, in the selection of grape cultivars for functional grape juice products, their physicochemical parameters, which contribute to sensory qualities, are of great concern. The physicochemical characteristics can be identified by analyzing TSS, Titratable acidity, pH value, color and Clarity [10].

Due to lack of detailed studies of physicochemical characteristics of Israel Blue grapes widely cultivated in Jaffna, Sri Lanka, this research study was a sole effort to explore the potentials for the introduction of a commercially viable beverage using locally grown Israel blue grapes

Correspondence
MMW Aponso
Department of Food Science and
Technology, Faculty of Applied
Sciences, University of Sri
Jaywardenepura, Sri Lanka

in Sri Lanka and compare its physicochemical properties with grape juices prepared using locally available imported grape varieties to Sri Lanka namely, Red Globe and Michele Palieri. The main objective of this study was to evaluate the physicochemical properties of Israel blue grape cultivar grown in Sri Lanka and assess its position compared to the imported varieties such as Red Globe and Michele Palieri which are widely available in the local market, in order to determine the suitability of Israel blue grapes to produce a commercially viable beverage by selecting the most suitable processing condition.

Materials and methods

Plant Materials

Fresh, ripen grapes without any visual blemishes, from the *Vitis vinifera* L. variety Israel blue, cultivated in the vicinity of Jaffna, North region of Sri Lanka were purchased from a generous farmer for the preparation of grape juices. The cultivar was grown on Pandhal trellis system in the vine yard. Fresh ripen Michele Palieri and Red Globe grapes without any visual blemishes were purchased from local supermarkets and the varieties were confirmed by the Department of Plant Quarantine, Sri Lanka.

Chemicals

Sodium hydroxide, sodium hypochlorite and phenolphthalein 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 to treat the grape pulp.

Sample preparation

Grape juice from three grape cultivars were subjected to an enzymatic treatment for further clarification according to a standard method with some modifications [11]. The best two treatment combinations that give the highest TSS/TA ratio were selected as "2 % pectinase enzyme, 40 °C incubation temperature, 2 hours incubation time" and "1.5 % pectinase enzyme, 40 °C incubation temperature, 2 hours incubation time" from a previous preliminary study. Grape juice of each variety were subjected to four different treatment methods namely raw juice, pasteurized juice, 1.5 % pectinase enzyme treated juice and 2 % pectinase enzyme treated juice as shown in Table 1. Raw juice was prepared by blending and converting grapes into a 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 11 μm. Pasteurized grape juice was prepared by pasteurizing the filtered raw juice under 90 °C for 5 minutes. Soon after pasteurization, juice was removed from the flame, filled into clean, previously washed and dried PET bottles and capped. To increase the effectiveness of the results, grape juice samples were prepared in triplicates and duplicate measurements were taken from each replicate. Hence, each grape variety consisted of 24 samples collectively. Grape juice samples were stored at (-18) °C for subsequent analysis.

Total Soluble Solids (TSS)

The brix values (TSS) of grape juice samples were measured using a portable hand held refractometer at room temperature. All the experimental measurements were replicated 3 times with duplicate measurements (n=6).

Titrateable Acidity (TA)

Titrateable acidity was determined according to the AOAC method [12]. A standard solution of 0.1N NaOH was prepared. Simultaneously 5 mL of grape juice was transferred to an Erlenmeyer flask, diluted up to the 50 mL graduation with distilled water and few drops (nearly 1ml) of phenolphthalein indicator was added. The sample was titrated against the standard NaOH solution to obtain an end point at pH 8.2 that gives a consistent pink color for 30 seconds. The following equation was used to determine the Titrateable Acidity (TA) as tartaric acid g/100 mL [13]. All the experimental measurements were replicated 3 times with duplicate measurements (n=6). Titrateable Acidity as tartaric acid

$$(\text{g}/100 \text{ mL}) = \frac{V_1 \times N \times 75 \times 100}{1000 \times V_2}$$

Where,

V_1 = Volume in ml of standard NaOH required for titration

N = Normality of the standard NaOH

V_2 = Volume in ml of the grape juice sample taken for the test

pH value

The digital pH meter (Hanna Instruments HI84435-01 Mini Titrator and pH Meter) was calibrated against standard buffer solutions. The grape juice samples were mixed well to homogenize and the pH values were measured using the calibrated pH meter. All the experimental measurements were replicated 3 times with duplicate measurements (n=6).

Color Analysis

Color of the grape juice samples and grapes from each variety were determined using a reflectance Chroma-Meter (Model CR-400, Konica Minolta Camera Co. Ltd, Osaka, Japan) based on the L^* (lightness or brightness), a^* (redness/greenness), b^* (yellowness/blueness) values and hue angle (H^0) according to a standard procedure [14]. The reflectance Chroma-Meter was standardized using a white plate and reflectance values of $Y = 93.93$, $x = 0.3131$, $y = 0.3189$ were used as standards. The equation, $[H^0 = \tan^{-1}(b^*/a^*)]$ was used to calculate the Hue angle (H^0). All the experimental measurements of grape juices were replicated 3 times with duplicate measurements (n=6). All the experimental measurements of grapes (fruits) were taken as a sample size of 10 from each grape variety under three directions (n=30).

Clarity

The clarity was determined according to a standard method [15]. The absorbance of the grape juice samples were measured at 660 nm using a UV-Vis Spectrophotometer (Model HACH-DR 6000). Distilled water was used as the reference. All the experimental measurements were replicated 3 times with duplicate measurements (n=6).

Results and discussion

Total Soluble Solids (TSS)

As shown in Table 1, there is a significant difference ($p < 0.05$) between brix values of raw juice, pasteurized juice, pectinase 1.5% treated juice and pectinase 2% treated juice. The mean brix values in grape juices vary from 12.25 – 18.75%; 11.33 – 15.83%; 15.25 – 19.83% in Israel blue grapes, Red Globe grapes and Michele Palieri grapes, respectively. Compared to the raw and pasteurized juices, the increment of Brix values in enzymatically treated juices is due to the presence of the pectinase enzyme. The increment of

brix value was noted with the increment of the concentration of pectinase enzyme. The increase in TSS is related to greater degree of tissue breakdown, releasing more compounds such

as sugars and conversion of insoluble pectin which contribute to soluble solids by pectinase enzyme [16-18].

Table 1: Effect of different treatments on brix values of grape varieties

Type of grape juice	TSS (%)		
	Israel Blue	Red Globe	Michele Palieri
Raw juice	12.25 ± 0.27 ^a	11.33 ± 0.26 ^a	15.25 ± 0.27 ^a
Pasteurized juice	12.83 ± 0.26 ^b	12.17 ± 0.26 ^b	16.17 ± 0.26 ^b
Pectinase 1.5% treated juice	15.67 ± 0.26 ^c	14.17 ± 0.26 ^c	18.17 ± 0.26 ^c
Pectinase 2% treated juice	18.75 ± 0.27 ^d	15.83 ± 0.26 ^d	19.83 ± 0.26 ^d

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

Titrateable Acidity (TA)

As shown in Table 2, Titrateable Acidity of all grape juice samples in Israel blue grapes and Red Globe grapes are significantly different (p < 0.05) from each other. With regards to Michele Palieri grape variety, 1.5 % pectinase enzyme treated grape juice and 2 % pectinase enzyme treated grape juice are not significantly different from each other as both grape juices share the same letter (a) under Tukey comparison test at (p < 0.05). But raw juice, pasteurized juice and enzymatically treated juice in general are significantly

different from each other. In each grape variety the ascending order of the variation of TA % is raw juice, pasteurized juice, Pectinase 1.5 % treated juice and Pectinase 2 % treated juice. The increase in TA % in pectinase treated juices could be due to the formation of galacturonic acid by enzymatic breakdown of pectin during the process [19]. The acidity has a significant impact on the sensory qualities and TA % above 0.85 % are considered too tart for juice [20]. There's no adverse TA % increment due to the application of pectinase enzyme.

Table 2: Effect of different treatments on Titrateable Acidity of grape varieties

Type of grape juice	TA (%)		
	Israel blue	Red Globe	Michele Palieri
Raw juice	0.58 ± 0.005 ^d	0.45 ± 0.010 ^d	0.34 ± 0.008 ^c
Pasteurized juice	0.62 ± 0.005 ^c	0.52 ± 0.007 ^c	0.38 ± 0.008 ^b
Pectinase 1.5% treated juice	0.67 ± 0.008 ^b	0.55 ± 0.011 ^b	0.39 ± 0.004 ^a
Pectinase 2% treated juice	0.72 ± 0.010 ^a	0.69 ± 0.010 ^a	0.40 ± 0.008 ^a

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

pH

Referring to Table 3, the pH value of all grape juices under Israel Blue grape variety are significantly different (p < 0.05) from each other. The enzymatically treated juices in Red Globe variety are not significantly different from each other but significantly different from raw and pasteurized juices. Raw and pasteurized juices under Michele Palieri grapes are not significantly different from each other but significantly different from 1.5% pectinase treated juice and 2% pectinase

treated juice separately. In each grape variety the ascending order of the variation of pH value is pectinase 2% treated grape juice, pectinase 1.5% treated grape juice, pasteurized juice and raw juice. With the increment of Titrateable Acidity in grape juices, the pH values were decreased due to the application of pectinase enzyme. The decrease of pH values may be due to the release of carboxyl groups and galacturonic acid from pectin [21].

Table 3: Effect of different treatments on pH values of grape varieties

Type of grape juice	pH		
	Israel Blue	Red Globe	Michele Palieri
Raw juice	3.92 ± 0.012 ^a	3.81 ± 0.012 ^a	4.17 ± 0.086 ^a
Pasteurized juice	3.80 ± 0.012 ^b	3.71 ± 0.015 ^b	4.11 ± 0.012 ^a
Pectinase 1.5% treated juice	3.66 ± 0.015 ^c	3.62 ± 0.012 ^c	4.03 ± 0.018 ^b
Pectinase 2% treated juice	3.61 ± 0.012 ^d	3.60 ± 0.008 ^c	3.72 ± 0.014 ^c

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

Color

L*, a*, b* are measurements of brightness, redness and yellowness, respectively. Hue angle values in accordance with main colors are recorded as Red purple (h = 0°), Red (h = 20.14°), Yellow (h = 90.00°), Green (h = 164.25°) and Blue (h = 237.53°) [22, 23]. Referring to Table 4, color parameters L* and hue angle values are significantly different from each other (p < 0.05) in grape juices under each variety. In all grape varieties, the ascending order of L* values is, 2% pectinase treated grape juice, 1.5% pectinase treated grape

juice, raw juice and pasteurized juice. The ascending order of hue angle (H°) is 2% pectinase treated grape juice, 1.5% pectinase treated grape juice, raw juice and pasteurized juice. High level of L* value indicates high brightness level and low color intensity as mentioned in [24]. Hue angle is computed using a* and b* values. Hue angle between 20.14° and 90° indicates the variation from red color to yellow color as mentioned in [23]. Low hue angle values indicate tendency towards red color and high hue angle values indicate tendency towards yellow color.

Table 4: Color parameters of grape juices from different juice varieties

Grape Juice	L*	a*	b*	Hue angle (H ⁰)
Israel Blue				
Raw juice	17.54±0.14 ^b	11.36±0.11 ^b	9.38±0.11 ^b	39.55±0.43 ^b
Pasteurized juice	24.54±0.37 ^a	12.34±0.15 ^a	12.38±0.12 ^a	45.09±0.29 ^a
Pectinase 1.5% juice	13.56±0.21 ^c	8.76±0.17 ^c	6.54±0.13 ^c	36.75±0.41 ^c
Pectinase 2% juice	10.69±0.24 ^d	11.37±0.21 ^b	4.55±0.17 ^d	21.79±0.86 ^d
Red Globe				
Raw juice	40.25±0.23 ^b	3.54±0.04 ^c	13.16±0.08 ^b	74.96±0.20 ^b
Pasteurized juice	42.76±0.16 ^a	1.12±0.36 ^d	7.74±0.10 ^d	81.76±0.34 ^a
Pectinase 1.5% juice	39.49±0.24 ^c	11.64±0.05 ^a	14.61±0.26 ^a	51.44±0.54 ^c
Pectinase 2% juice	37.71±0.19 ^d	11.30±0.05 ^b	11.10±0.07 ^c	44.48±0.28 ^d
Michele Palieri				
Raw juice	21.23±0.05 ^b	10.87±0.09 ^d	7.71±0.10 ^b	35.35±0.24 ^b
Pasteurized juice	34.27±0.10 ^a	11.35±0.05 ^c	9.28±0.05 ^a	39.25±0.16 ^a
Pectinase 1.5% juice	20.63±0.08 ^c	11.50±0.07 ^b	7.30±0.07 ^c	32.40±0.39 ^c
Pectinase 2% juice	16.78±0.13 ^d	14.51±0.08 ^a	7.10±0.06 ^d	26.08±0.11 ^d

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

With regards to the color parameters of the grapes (fruit) as shown in Table 5; in the Israel blue grape variety; the highest and lowest L* values were recorded as means of 24.54 and 10.69 and the highest and lowest H⁰ values were recorded as means of 45.09 and 21.79 respectively. In the Red Globe grape variety; the highest and lowest L* values were recorded as means of 42.76 and 37.71 and the highest and lowest H⁰ values were recorded as means of 81.76 and 44.48 respectively. In the Michele Palieri grape variety; the highest and lowest L* values were recorded as means of 34.27 and 16.78 the highest and lowest H⁰ values were recorded as means of 39.25 and 26.08 respectively. When considering all grape varieties, the highest and lowest L* values were recorded as means of 42.76 (Red Globe pasteurized grape juice) and 10.69 (2% pectinase treated Israel Blue grape juice)

and the highest and lowest H⁰ values were recorded as means of 81.76 (Red globe pasteurized grape juice) and 21.79 (2% pectinase treated Israel blue grape juice), respectively. Referring to Table 5, the highest and lowest L* values were recorded as means of 28.95 (Red Globe grape variety) and 27.18 (Israel Blue grape variety), respectively and the highest and lowest H⁰ values were recorded as means of 26.33 (Red globe grape variety) and 12.73 (Michele palieri grape variety), respectively. Israel Blue grapes hold a color in red-purple to red color region being much closer to red color; Michele Palieri grapes hold a color in red-purple to red color region being much closer to red-purple color and Red Globe grapes hold a color in red to yellow color region being much closer to red color, in accordance with the hue angle values [22, 23].

Table 5: Color parameters of grapes (fruit) from different grape varieties

Color parameters	L*	a*	b*	H ⁰
Israel Blue grapes	27.18 ± 0.74 ^c	4.69 ± 0.25 ^b	1.41 ± 0.13 ^b	17.29 ± 1.54 ^b
Red Globe grapes	28.95 ± 0.74 ^a	5.90 ± 0.57 ^a	2.71 ± 0.21 ^a	26.33 ± 2.52 ^a
Michele Palieri grapes	28.28 ± 0.49 ^b	1.56 ± 0.14 ^c	0.34 ± 0.02 ^c	12.73 ± 0.98 ^c

*Data presented as mean values 10 grapes samples from each variety under 3 directions ± SD (n=30). a,b,c, letters in the same column are significantly different at (p < 0.05) level.

Clarity

As shown in Table 6, there is a significant difference in absorbance values between different types of grape juices and between different grape varieties (p<0.05). Lowest absorbance level at 660 nm wave length indicates highest degree of clarity in grape juices. As shown in Table 6, the descending order of absorbance values indicate ascending order of degree of clarity. The ascending order of degree of clarity in grape juices is; raw juice, pasteurized juice, 1.5% pectinase treated juice and 2% pectinase treated juice in all grape varieties. The increase in enzymatic concentration increase the rate of clarification by reducing electrostatic repulsion between cloud particles which causes the particles to aggregate into larger particles and eventually settle down [25]. The clarity of pasteurized juices were greater than the raw juices as the temperature increases the rate of clarification [26].

Clarity showed the lowest absorbance values at highest enzyme concentration, where lower absorbance indicates a clearer juice is being produced [26]. The lowest absorbance value in Israel Blue grape variety was recorded as a mean of 0.039 in 2 % pectinase treated grape juice and the highest value was recorded as a mean of 0.310 in pasteurized juice. The lowest absorbance value in Red globe grape variety was recorded as a mean of 0.045 in 2 % pectinase treated grape juice and the highest value was recorded as a mean of 0.616 in pasteurized juice. The lowest absorbance value in Michele Palieri grape variety was recorded as a mean of 0.016 in 2 % pectinase treated grape juice and the highest value was recorded as a mean of 0.202 in pasteurized juice. When considering all grape varieties, the most clarified juice is 2 % pectinase treated Michele Palieri grape juice and the least clarified juice is Red globe raw grape juice.

Table 6: Results of clarity based on absorbance at 660 nm

Type of grape juice	Israel Blue grapes	Red Globe grapes	Michele Palieri grapes
Raw juice	0.310 ± 0.005 ^a	0.616 ± 0.004 ^a	0.202 ± 0.001 ^a
Pasteurized juice	0.227 ± 0.008 ^b	0.243 ± 0.003 ^c	0.128 ± 0.002 ^b
Pectinase 1.5% treated juice	0.047 ± 0.001 ^c	0.082 ± 0.003 ^b	0.029 ± 0.003 ^c
Pectinase 2% treated juice	0.039 ± 0.003 ^d	0.045 ± 0.003 ^d	0.016 ± 0.003 ^d

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

Conclusion

The present study concluded that the pectinase enzyme treatment increases the TSS levels, TA levels, pH, and clarity in grape juices compared to raw juice and pasteurized juice. It enables enhancement of physicochemical properties of grape juice which in turn improves the juice quality produce grape juice as a commercially viable beverage. Based on the comparative study between Israel blue, Red globe and Michele Palieri grape cultivar; 2 % pectinase enzyme treated Israel blue grape juice shows the highest values of TA; moderate values of TSS, pH, clarity and lowest value for Hue angle which is approximately in the red color region. Therefore, the study confirms, the selected pectinase enzymatic treatment increases the suitability of Israel blue grapes to produce a commercially viable grape juice.

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