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Physicochemical and Sensory Evaluation of Non-alcoholic Wine-like Beverages Prepared from Selected Grape Cultivars

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Abstract

The heat treatment of holding grape mash at 60°C for 30 minutes allowed recovery of a higher concentration of specific phenolic compounds into juice without giving caramelized flavours and odours. Physicochemical and sensory properties of four grape juice blends prepared from three selected grape cultivars, 'Castel 19637', 'Lucie Kulman', 'Sovereign Coronation' and water were evaluated on the selection for a non-alcoholic wine-like beverage. A descriptive sensory study was conducted using 18 trained panelists to assess the color, sweetness, astringency, viscosity and overall acceptability of the beverages. Overall, considering the sensory attributes, concentration of bioactive phenolics, antioxidant capacity measured by ferric reducing antioxidant capacity, and moderate sugar content, the blend of 'Castel 19637': water (3:2, v:v) was found to have more potential to be used in developing a wine-like functional beverage. 'Castel 19637', which was originally developed as a red wine grape cultivar, can be recommended as a suitable grape cultivar for developing a functional beverage.

Keywords: Grape beverages; Descriptive sensory analysis; Antioxidant capacity; Phenolics

Introduction

In North America the market for functional beverages such as enhanced water, a non-caloric source of vitamins and minerals, increased by 73% from 2002 to 2007 [1]. A market survey revealed that sixty percent of consumers who purchased functional beverages chose them in part for the presence of antioxidants [1]. Phenolics are physiologically active compounds with antioxidant properties which are abundant in fruits and vegetables and could provide significant beneficial health effects [2]. Phenolics possess the ability to prevent various diseases associated with oxidative stress, including cancers, cardiovascular and neurodegenerative diseases [2,3].

Grapes are one of the fruits that contain the highest content of phenolic compounds [4]. In the preparation of grape juice, grapes are pressed first into a mash. Heat application to the grape mash was reported to be an effective pre-treatment for recovering more anthocyanins and stilbenes in the juice than direct pressing without a heat pretreatment [5]. Fuleki and Ricardo-Da-Silva [6] reported that flavan-3-ols concentrations of juices prepared from the wine grape cultivars 'Baco noir' and 'Marechal Foch' increased when the respective mashes were heated at 60°C for 60 minutes before pressing. However, the impact of this heat treatment on the sensory qualities of the juice was not reported in the above study. The pressing had more of a positive effect on the final concentration of flavan-3-ols in the juice than did the cultivar effect [6]. However, grape flavor is more likely to be adversely impacted by heat processing than the pigments and phenolics of grapes [7,8].

This study sought to develop a non-alcoholic wine-like beverage from grape juice blends. The objective was to prepare blends that were 'wine-like' in appearance, with an accompanying astringency and tartness similar to grape wine and having high antioxidant content. The effectiveness of a heat pre-treatment on the bioactive content, physicochemical, and quality of grape juice was examined. In addition, several grape juice blends from selected grape cultivars were prepared and analyzed for bioactive concentration, antioxidant capacity, and sensory properties.

Materials and Methods

Plant materials and chemicals

'Castel 19637' was obtained from the John Warner Farm of Kentville, NS, Canada while 'Lucie Kuhlman' and 'Sovereign Coronation' were obtained from Stonehill Fruit and Juice Company, Kingston, NS, Canada. From each cultivar, twenty kilograms of fruits at commercial maturity level were harvested in between late September and early October, 2009 and used in juice preparation. The 'Sovereign Coronation' grapes were used in the optimization of the heating treatment of grape mash. A commercial grape juice product that was purchased from the local market was used for the comparison purposes.

The liquid chromatography standards of sugars and organic acids were purchased from Sigma-Aldrich Ltd. (Oakville, ON, Canada). The phenolic standards were purchased as follows: malvidin-3-O-glucoside (M3G) from Chromatographic Specialities Inc. (Brockville, ON, Canada); petunidin-3-O-glucoside (P3G) and delphinidin-3-O-glucoside (D3G) from Polyphenols Laboratories (Sandnes, Norway); cyanidin-3-O-glucoside (C3G) from Extrasynthese (GenayCedex, France); catechin, epicatechin, epigallocatechin, epigallocatechingallate, epicatechingallate, quercetin (Q) quercetin-3-O-glucoside (Q3Glu), resveratrol and resveratrol glucoside from ChromaDexInc (Santha Ana, CA, USA); quercetin-3-O-rhamnoside

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(Q3R) and quercetin-3-O-galactoside (Q3Gal) from Indofine Chemical Company (Hillsborough, NJ, USA).

Heat treatment of grape mash

Grapes were detached from the stems, washed and drained. A sample of 150 g was cut into slices (2-4 mm) (Vegetable chopper Model EHC 650, Black & Decker Corp, Towson, ML, USA) and heated in a convection oven. Temperature-time treatments used were: i) 60°C; 30 min, ii) 60°C; 60 min, iii) 85°C; 30 min; iv) 85°C; 60 min. Immediately after heating, the grape juice was recovered by the use of a stainless steel fruit squeezer (Norpro Inc., Everette, WA, USA); the juice was then filtered through four layers of cheese cloth. The filtered juice was subjected to physiochemical, bioactive concentrations and antioxidant capacity analysis in order to determine the optimum heat pre-treatment.

Preparation of the grape juice blends

Previous evaluation of physicochemical characteristics (titratable acidity, total soluble solids, and concentration of phenolics) of the juice and fresh fruits of ten Nova Scotia grown grape cultivars showed that 'Castel 19637', 'Lucie Kuhlman' and 'Sovereign Coronation' had bioactive phenolic compositions that showed promise for use in functional beverage formulation [9]. Grapes from these cultivars were subjected to the heating regime previously found to yield the highest bioactive content in the resultant juice (65°C; 30 minutes). Using a wine pressing ratchet (Musca Wine Pressing and Supplies Limited, Ottawa, ON), 20 kg of sliced, heat-treated grapes was pressed. The juice was filtered and pasteurized using a batch pasteurization method (72°C; holding time of 1.5 minutes). The juice samples were hot-filled into sterilized containers and stored (4°C) for less than ten days before use for further analysis.

Based on the results of preliminary investigations, four grape juice blends were prepared by mixing grape juice of three cultivars and potable water according to the proportions as mentioned below:

Blend A: 'Castel 19637': water (3:2, v:v);

Blend B: 'Castel 19637': 'Sovereign Coronation': water (2:1:0.5, v:v:v);

Blend C: 'Castel 19637': 'Lucie Kuhlman': water (2:1:1, v:v:v); and Blend D: 'Castel 19637': 'Lucie Kuhlman': 'Sovereign Coronation': water (2:1:1:0.25, v:v:v).

Physicochemical characteristics of grape juice blends

Total soluble solids (TSS, °Brix) were measured using a bench model refractometer (Model 300016, Sper Scientific Ltd, Scottsdale, AZ, USA). Titratable acidity was measured using a semi automatedtitrator (DMP 785, Metrohm Ltd, Herisau, Switzerland). The titration involved the use of 0.1 M NaOH as the titrant to the pH 8.2 end point. The titratable acidity was expressed as % Titratable Acidity (TA), expressed in terms of tartaric acid, the predominant acid in grapes [10]. Colour of the beverages was determined by using a reflectance colorimeter (Model CR-300, Minolta Camera Co. Ltd, Osaka, Japan) and reported as L*, a*, and b* values [11]. All of the above parameters were determined in both heat treated juices and grape juice blends. The common sugars in grape juice (sucrose, glucose, and fructose) were analyzed by ion exchange chromatography using an HPLC system (Model Beckman Gold, Beckman Coulter Inc., Fullerton, CA, USA) equipped with a Aminex HPX-87P column (300 mm \times 7.8 mm, 5 $\mu m;$ 30°C) (Bio-Rad Laboratories Inc., Hercules, CA, USA), a Carbo-P guard column (Bio-Rad Laboratories Inc., Hercules, CA, USA), and a refractive index (RI) detector (Waters 2414, Waters Corp., Milford, MA, USA). The stationary phase of nanopure water (0.6 mL/min) was run with total run time of 30 minutes. The retention time for sucrose, glucose and fructose were 13.1, 15.4, and 22.2 minutes, respectively. The concentrations of standards which were used were: 0-30 mg/mL of sucrose; 0-20 mg/mL of glucose and 0-16 mg/mL of fructose.

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Organic acids analysis was performed using the method of Soyer et al. [12]. A HPLC system (Model Beckman Gold, Beckman Coulter Inc., Fullerton, CA, USA) equipped with a Synergi Hydro-RP (250 mm × 4.6 mm, 4µm) column (Phenomenex Inc., Torrance, CA, USA) and a C-18 guard column (Phenomenex Inc., Torrance, CA, USA), that were held at 30°C were used in the analysis. The mobile phase was 0.01 M H_2SO_4 at pH 2.5 with a flow rate of 0.5 mL/min and the total run time of 30 minutes. The retention times for tartaric, malic and citric acids were 6.10, 7.50, and 13.10 minutes, respectively. The linear range of standards were 0-6.03 mg/mL, 0-9.18 mg/mL, and 0-1.01 mg/ mL for tartaric, malic and citric acids, respectively. All the analyses were done in triplicate.

Concentrations of phenolics and antioxidant capacity

The phenolic content of the heated mash and the juice blends was evaluated in terms of the major phenolic sub-classes (flavonols, flavan-3-ols, anthocyanins, and stilbenes). Major phenolics present were identified and quantified using HPLC-ESI-MS/MS as described by Rupasinghe et al. [13].

The ferric reducing antioxidant power assay (FRAP), a measure of antioxidant capacity, was performed using an established method [14] with modifications as described by Rupasinghe et al. [13]. Pigments were determined as total anthocyanin concentration by the pH–differential method (AOAC method 2005.02). Results were expressed as mg of malvidin-3-O-glucoside equivalents per liter of juice. All the analyses were done in triplicate.

Sensory evaluation of juice blends

For the sensory analysis component of this work, a sample of convenience consisting of student and staff members of the campus was used. Potential panelists were screened for their ability to detect sweetness, sourness, astringency, and texture [15]. A group of 18 panelists (8 male and 10 female; ranging in age from 19-50 years) had the requisite sensory acuity. Panelists were further trained to rate sweetness, sourness, astringency, and viscosity on unstructured scales using the reference points suggested by Meilgaard et al. [15]. The method of Poste et al. [16] was used for rating viscosity. The 'wine-like color' was categorized by both hue and intensity by having panelists look through a 6 cm depth of liquid held against a white background. A reference sample of a red wine (Merlot, Donini Collection, Milano, Italy) was used.

Descriptive sensory analysis of the experimental juice blends and the commercial grape juice were performed by the trained panelists. A 15 cm unstructured scale, ranging from 'not' to 'very' was used for each of the attributes identified above. Samples were assigned three digit codes, and presented to panelists in a balanced, randomized manner. Panelists were asked to evaluate color first, followed by astringency, sourness, sweetness, and then viscosity. The sensory data from the descriptive testing was subjected to analysis of variance, blocked by panelist. The assumption of normal distribution was confirmed as described by Montgomery [17]. For the ANOVA, the general linear model (GLM) procedure of SAS 9.1 was used. The Duncan's Multiple Range Test at a level of $p \le 0.05$ was used to detect significant differences between means.

Results and Discussion

Effect of heat-treatment of grape mash on phenolics of juice

In general, heat treatment of grape mash increased the TSS and the TA of the resultant juice relative to the juice extracted from an unheated control (Table 1). The extent and duration of the heat treatment was directly proportional to increases in TSS, and to a lesser extent to an increase in TA. In spite of these differences in TSS and TA, there was no difference in TSS/TA ratio among the different heat treatments (Table 1). The heat treatment prior to juice extraction also resulted in increased concentration of total anthocyanins (Table 1). The most extensive heat treatment (85° C, 30 min) exhibited a 26fold increase in total anthocyanins present in the juice relative to the unheated control (p=0.0001). Quantification of individual classes of phenolics showed that heating the mash at 65°C, 30 min or 85°C, 15 min resulted in greater concentrations of total flavan-3-ols, flavonols, and stilbene being extracted into the juice compared to the unheated control (Figure 1).

Of the temperature-time pre-treatments examined in this study, heating the grape mash to 65°C for 30 minutes before pressing resulted in an organoleptically acceptable juice with significantly higher levels of total flavan-3-ols, flavonols, and stilbenes. Although juice obtained after the heat treatment at 85°C for 30 min contained a higher concentration of the phenolic compounds examined, preliminary studies indicated that the organoleptic quality of this juice was unacceptable due to extremely sour and caramelized flavor (data not present). Similarly, Morris et al. [18] reported that higher extraction temperatures of 85°C and 99°C yielded grape juice of more intense color than that extracted at 60°C. In addition, juice extracted at high temperatures has been shown to have a greater tendency to turn brown during storage [18]. Taking into consideration both sensory, phenolics and antioxidant capacity, the heat treatment of 65°C and 30 min was selected and used in the preparation of juice for blends and the sensory test.

Physicochemical properties of grape juice blends

In the four juice blends, TSS ranged from 13.3 to 18.2 while TA ranged from 0.75 to 1.15. The variation in TSS/TA was from 15.3 to 17.7 (Table 2). Compared to the other blends, Blend A (Castel 19637': water; 3:2, v:v) possessed the lowest amounts of total quantified sugar, TSS and TA (Table 2). However, Blend A possessed the highest TSS/TA ratio. There was no significant difference in total quantified anthocyanin content among the blends; the commercial juice possessed a significantly lower concentration of anthocyanin (Table 2). Blend C possessed lower L* value than the Blend B, which means that the colour intensity of Blend C was higher than that of Blend B (Table 2).

When considering the concentrations of phenolic sub-classes, all four blends studied had similar concentrations of quantified flavonols, flavan-3-ols and anthocyanins and all had significantly higher levels of flavonols and flavan-3-ols than that of the commercial grape juice (Table 3). The antioxidant capacity, as measured using the FRAP assay,

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Grape	TSS TA (°Brix) (%	ΤΔ 1	TSS/TA ratio		Instrumental Colou	ır	Total anthocyanin concentration ² (mg M3GE/L)	FRAP ³ (g TE/L)
Mash Treatment		(%)		L*	a*	b*		
Control⁴	16.1c	0.60c	26.6	42.3 <i>a</i>	24.4 <i>b</i> c	25.6 <i>a</i>	9.1 <i>d</i>	0.15c
65 °C-15 min	17.1 <i>b</i>	0.68 <i>bc</i>	25.5	28.6b	33.0 <i>a</i>	18.5 <i>b</i>	28.4c	0.30c
65 °C-30 min	17.5b	0.74 <i>b</i>	23.7	23.7c	29.2 <i>ab</i>	12.4c	57.5b	1.62 <i>b</i>
85 °C-15 min	17.5b	0.79 <i>ab</i>	22.4	23.5c	27.8ab	11.7c	67.2b	1.66 <i>b</i>
85 °C-30 min	18.9 <i>a</i>	0.89 <i>a</i>	21.7	21.4c	20.8c	6.3 <i>d</i>	235.6 <i>a</i>	4.05 <i>a</i>
P-value⁵	0.0001	0.0023	0.0668	0.0001	0.0059	0.0001	0.0001	0.0001

TSS, Total soluble solids

¹TA, Percentage titratable acidity (g tartaric acid equivalence/100 mL)

²Total anthocyanin content measured using pH differential method (M3GE, malvidin-3-O-glucoside equivalence)

³FRAP, Ferric reducing capacity of plasma, g TE/L, g Trolox equivalence per L

⁴Control consisted of juice obtained by pressing grapes without subjecting to the heat treatment of grape mash

⁵Means (n=3) followed by different letters (a-d) within the same column represent significant differences according to Duncan's Multiple Range test (p<0.05)



Figure 1: Effect of heat treatments of grape mash on total quantified flavan-3-ols, flavonols and stilbenes (A), and total quantified anthocyanins (B) concentrations of juices prepared from 'Sovereign Coronation' grapes. (n=3).

was higher in Blend C than in the Blend B but similar to that in Blend A and D.

Sensory attributes of grape juice blends

The responses of the panelists in the descriptive analysis of the five juice samples are presented in Table 4. There was no significant difference in panelists' responses for hue and colour intensity of the four blends. The panelists could not distinguish the colour difference between Blend C and Blend B that was apparent based on the instrumental lightness (L*) values (Table 2). Sensory scores for the sweetness of blends ranged from 6.7-8.2 on a scale of 15 (Table 4). Blends A and B were given significantly lower scores for sweetness than the commercial juice by the panelists, suggesting that it was more wine-like in this attribute. According to the panelists, Blend A exhibited significantly lower sourness than in Blend B (Table 4). The results were verified by chemical analysis; TA of Blend A was lower than that of in the Blend B. The perception of astringency in Blend D was significantly higher than that of Blend A (Table 4). Phenolic compounds, especially flavan-3-ol polymers, as well as organic acids, contribute to the astringency of wine [19]. In the present study, only

monomers of flavan-3-ol were quantified and there was no variation observed in the flavan-3-ols concentrations of four blends. When rated for overall organoleptic acceptability, no significant difference was found between the blends (Table 4).

Conclusions

Heating grape mash at 65°C for 30 min before pressing increased the concentrations of anthocyanins, flavan-3-ols, flavonols and stilbenes by over 6-fold in juice when compared to the unheated control. Four grape juice blends were prepared in an attempt to identify a non-alcoholic, wine like, bioactive phenolics-rich beverage with acceptable sensory attributes. Overall, considering sensory, bioactive phenolics and antioxidant capacity, and moderate sugar content, the blend of 'Castel 19637': water (3:2, v:v) was found to have more potential to be used in developing a wine-like functional beverage. The 'Castel 19637' was originally developed and established as a red wine grape cultivar in Nova Scotia; however, the results indicate that this cultivar is also suitable for non-alcoholic grape juice manufacturing. This study also suggest that recovery of bioactive phenolics from grape pomace can be achieved by a simple, less expensive and environmental

Table 2: Physicochemical characteristics of grape juice blends and a commercial product.

Grape juice beverages	TSS (ºBrix)	Total quantified sugar ¹ (mg/mL)	TA² (%)	Total quantified acid ³ (mg/L)	TSS/TA	Total quantified anthocyanin⁴ (mg M3GE/L)	Instrumental Colour		
							L*	a*	b*
Blend A⁵	13.3e	110c	0.75d	19.7 <i>bc</i>	17.7 b	24.8 <i>a</i>	15.7 <i>bc</i>	10.7 <i>a</i>	6.8 <i>ab</i>
Blend B ⁶	17.1 <i>b</i>	130 <i>b</i>	1.12a	21.1 <i>ab</i>	15.3 <i>d</i>	24.2a	16.0 <i>ab</i>	8.77b	7.2a
Blend C ⁷	18.2 <i>a</i>	150 <i>a</i>	1.15a	18.4c	15.8 <i>d</i>	24.5 <i>a</i>	15.3c	9.42 <i>b</i>	5.9c
Blend D ⁸	16.6c	148 <i>a</i>	0.99 <i>b</i>	21.7c	16.7 <i>c</i>	24.9 <i>a</i>	15.5 <i>bc</i>	7.64c	6.0 <i>bc</i>
Commercial grape juice	16.3 <i>d</i>	130 <i>b</i>	0.81c	16.1 <i>d</i>	20.3 <i>a</i>	5.4 <i>b</i>	16.40 <i>a</i>	8.77b	6.7 <i>ab</i>
P-value9	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0118	0.0043	0.0035

TSS, Total soluble solids

¹Sum of the concentrations of glucose and fructose quantified by HPLC.

²TA, Percentage titratable acidity (g tartaric acid equivalence/100 mL)

³Sum of the concentrations of tartaric, malic and citric acids quantified by HPLC.

⁴ Total anthocyanin concentration measured using pH differential method (M3GE, malvidin-3-O-glucoside equivalence)

⁵Blend A: 'Castel 19637' : water (3:2; v:v) ⁶Blend B: 'Castel 19637': 'Sovereign Coronation' : water (2:1:0.5; v:v:v)

⁷Blend C: 'Castel 19637': 'Lucie Kuhlman': water (2:1:1; v:v:v)

⁸Blend D: 'Castel 19637': 'Lucie Kuhlman': 'Sovereign Coronation': water (2:1:1:0.25; v:v:v)

⁹ANOVA P-values showing the effect of juice products on the considered variables

Means (n=3) followed by different letters (a-d) within the same column represent significant differences according to Duncan's Multiple Range test (p<0.05).

Table 3: Concentration of selected bioactive phenolics and antioxidant capacity of grape juice blends.

Grape juice beverages	Total quantified flavonols (mg/L) ¹	Total quantified flavan-3-ols (mg/L) ²	Total quantified anthocyanins (mg/L) ³	Total quantified flavonoids (mg/L) ⁴	FRAP⁵ (g TE/L)
Blend A ⁶	0.25b	0.10 <i>b</i>	18.20 <i>a</i>	18.6 <i>a</i>	2.44 <i>bc</i>
Blend B ⁷	0.26 <i>b</i>	0.13 <i>b</i>	17.88 <i>a</i>	18.3 <i>a</i>	2.25c
Blend C ⁸	0.26 <i>b</i>	0.14 <i>b</i>	18.06 <i>a</i>	18.3 <i>a</i>	2.54b
Blend D ⁹	0.28 <i>b</i>	0.20 <i>b</i>	17.45 <i>a</i>	17.9 <i>a</i>	2.39 <i>bc</i>
Commercial grape juice	1.86 <i>a</i>	1.10 <i>a</i>	6.96 <i>b</i>	9.9 <i>b</i>	5.15 <i>a</i>
P-value ¹⁰	<0.0023	<0.0006	0.004	0.0015	<0.001

¹Sum of the concentrations of Q-3-O-glucoside and Q-3-O-galactoside

²Sum of the concentrations of cathechin, epicathechin and epigallocatechin

³Sum of the concentrations of malvidin-3-Oglucoside, delphinidin-3-O-glucoside, petudin-3-Oglucoside and cyanidin-3-O-glucoside.

⁴Sum of the concentrations of total quantified flavonols, total quantified flavan-3-ols and total quantified anthocyanins.

⁵FRAP, Ferric reducing capacity of plasma, g TE/L, g Trolox equivalence per L

⁶Blend A: 'Castel 19637' : water (3:2; v:v) ⁷Blend B: 'Castel 19637': 'Sovereign Coronation': water (2:1:0.5; v:v:v)

⁸Blend C: 'Castel 19637': Lucie Kuhlman': water (2:1:1; v:v:v)

Blend D: 'Castel 19637': Lucie Kuhlman': 'Sovereign Coronation': water (2:1:1:0.25; v:v:v)

¹⁰ANOVA P-values showing the effect of juice products on the considered variables

Means (n=3) followed by different letters (a-c) within the same column represent significant differences according to Duncan's Multiple Range test (p<0.05)

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Table 4: Descriptive sensory analysis of grape juice beverages by trained panelists.

Grape Juice beverages	Sensory attribute ¹									
	Colour		Sweetness	Courseas	Astringanov	Viccosity				
	Hue	Intensity	Sweethess	Sourness	Astingency	VISCOSILY				
Blend A ²	11.0	11.4	6.7c	8.1 <i>b</i>	7.7b	4.0	8.6			
Blend B ³	11.0	11.3	7.3 <i>bc</i>	9.7 <i>a</i>	8.9 <i>ab</i>	3.9	7.7			
Blend C ⁴	11.2	11.9	8.1 <i>ab</i>	8.6 <i>ab</i>	8.6 <i>ab</i>	3.6	8.8			
Blend D⁵	11.4	11.3	8.2 <i>ab</i>	8.7 <i>ab</i>	9.2 <i>a</i>	3.8	8.0			
Commercial grape juice	11.6	10.9	9.1 <i>a</i>	5.2c	5.4c	3.3	8.4			
P-value ⁶	0.7676	0.3397	0.0072	0.0001	0.0001	0.6811	0.7405			

¹Descriptive panel evaluation of attributes on 15 cm scale.

²Blend A: 'Castel19637': water (3:2; v:v) ³Blend B: 'Castel19637': 'Sovereign Coronation' : water (2:1:0.5; v:v:v)

⁴Blend C: 'Castel19637': 'Lucie Kuhlman': water (2:1:1; v:v:v)

⁵Blend D: 'Castel19637': 'Lucie Kuhlman': 'Sovereign Coronation': water (2:1:1:0.25; v:v:v)

6ANOVA P-values showing the effect of juice products on the considered variables

Means (n=18) followed by different letters (a-c) within the same column represent significant differences according to Duncan's Multiple Range test (p<0.05).

friendly technique such as heat treatment of pomace followed by press. Dehydration of juice by spray drying or other technique could result in development of food and nutraceutical ingredients.

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