# EFFECT OF THE ADDITION OF FRUIT JUICES ON GRAPE MUST FOR NATURAL BEVERAGE PRODUCTION

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ABSTRACT

The consumer attention for products with healthy properties is increased in time, and fruit juices, for their ease of consumption, can satisfy this demand providing them bioactive compounds. The grape juice has numerous health benefits demonstrated by several studies such as, among other, the antioxidant activities and the positive functions of their phenolic compounds. This work is aimed at blending grape and others fruits in a new fruit juice made only with natural ingredients of local production. The grape juice (cv Barbera) has substituted water and its percentage was fixed (70%). It was mixed with apple (cv Golden delicious), pear (cv Williams) and peach (cv Red Haven) juices to obtain 25 different prototypes. In each of these at least two fruit juices were present and added in a percentage variable from 0 to 25%, with a step of 5%. The objectives of this study were to check the feasibility of the mixing process and the evaluation of the samples overall pleasantness.

Other sensory aspects of samples were also evaluated by consumers with a JAR (just-about-right) structured scale. The results didn't reveal particular technological problems regarding the blending process. The Brix mean value of the samples was about 15.3, with a significant reduction compared to that of the grape juice (about 19). The pH mean value of the samples (3.44) was significantly higher than that of the grape juice (3.36). The titrable acidity and the antioxidant capacity mean value of the samples was, namely, 6.22 g $L^{-1}$ and 535.18 mg $L^{-1}$. The penalty analysis of the liking test pointed out the importance of the persistence in mouth. The overall pleasantness was significantly ($p \leq 0,01$) positively correlated with the °Brix/acid ratio (r=0.54) and samples with the highest percentage of pear juice were generally preferred.

- Keywords: fruit, grape must, health, juice, sensory analysis -

## INTRODUCTION

Fruit consumption has a positive impact on health (O'NEIL *et al.*, 2011) and, including also vegetables, five are their daily servings (FSA, 2010), though this advice is generally ignored (WOOTTON-BEARD and RYAN, 2011). In this regard, the "Dietary Guidelines for Americans" consider the 100% fruit juice as alternative to whole fruit (USDA, 2010). Indeed, fruit juices in general are deemed as one of the main sources of bioactive compounds for diet (RODRÍGUEZ-ROQUE *et al.*, 2014). Even if the link between weight and sweetened beverages, including fruit juice 100%, must to be taken into account, referring to these latters, there is no consistent association (O'NEIL *et al.*, 2011) and, actually, these have demonstrated to improve nutrient adequacy among children and adolescents of 2-18-year-olds (O'NEIL *et al.*, 2012). Also grape has proved to have numerous health benefits, such as antioxidant activity and the functions of flavonoid compounds (VISLOCKY and FERNANDEZ, 2010; WOOTTON-BEARD and RYAN, 2011). Grape-based products may prevent cardiovascular deseases, decrease oxidative stress and protect against atherosclerosis. Results from animal models suggest that especially purple grape juice more effectively improves blood lipids (VISLOCKY and FERNANDEZ, 2010). From a organoleptic and sensory perspective, grape juice is characterised by a high concentration of sugars and acids, a low pH and, generally, a very poor odour/aroma. Thus, grape juice has a high-energy value, which reduces the nutritional, while its high acidity and low odour/aroma intensity can reduce consumer preference. OJEDA *et al.* (2009) highlighted the too high sugar content of the pure grape juice and, for this reason, it is important to reach a right sugar/acids balance to develop appreciable grape juice. To reach this result it is necessary to use the optimal grape variety and/or mixing it with other fruit. The blending, indeed helps to improve flavour, taste, and nutritive value and it reduces the cost of production, improves storability and inhibits microbial growth (BHARDWAJ and PANDEY, 2011). As reported by BATES and MORRIS (2001), the reasons for producing blends are many and all attributable to adjust and improve acceptability. The aim of this work was to develop an innovative concept of fruit juice obtained by mixing grape with other fruit juices to reduce its sugar concentration, acidity and to improve its olfactory profile. The tested fruit juices (peach, pear and apple) were chosen based on their appreciation by consumers, low acidity and sugar content, high antioxidant activity and high odour/aroma intensity. The use of grape must would also help to reduce the wine surplus that, currently, amounts to, approximately, 30 million hectolitres world wide (RAMOS *et al.*, 2012; AYLWARD, 2012).

## MATERIALS AND METHODS

### Juice production

The grape juice (cv Barbera) was provided by Terre dei Santi (Castelnuovo Don Bosco, Asti, Italy), while the other fruit juices were provided by Valter Valle Farm (San Damiano d'Asti, Asti, Italy). The apple, pear and peach juices were obtained from the Golden delicious, Williams and Red Haven cultivars, respectively. For juice production, fruits were directly pressed, and the juice was filtered and stored at +1°C until use. Because the aim of this study was to develop a new grape-based juice, the percentage of grape juice was fixed (70%) and the other fruit juices were added in percentages from 0 to 25%, with a step of 5% (Table 1).

Table 1 - Experimental plan of blending.

| Sample code | Barbera juice (%) | Fruit juices (%) | | |
|---|---|---|---|---|
| | | Pear | Peach | Apple |
| S-1 | 70 | 0 | 5 | 25 |
| S-2 | 70 | 0 | 10 | 20 |
| S-3 | 70 | 0 | 15 | 15 |
| S-4 | 70 | 0 | 20 | 10 |
| S-5 | 70 | 0 | 25 | 5 |
| S-6 | 70 | 5 | 0 | 25 |
| S-7 | 70 | 5 | 5 | 20 |
| S-8 | 70 | 5 | 10 | 15 |
| S-9 | 70 | 5 | 15 | 10 |
| S-10 | 70 | 5 | 20 | 5 |
| S-11 | 70 | 5 | 25 | 0 |
| S-12 | 70 | 10 | 0 | 20 |
| S-13 | 70 | 10 | 5 | 15 |
| S-14 | 70 | 10 | 10 | 10 |
| S-15 | 70 | 10 | 15 | 5 |
| S-16 | 70 | 10 | 20 | 0 |
| S-17 | 70 | 15 | 0 | 15 |
| S-18 | 70 | 15 | 5 | 10 |
| S-19 | 70 | 15 | 10 | 5 |
| S-20 | 70 | 15 | 15 | 0 |
| S-21 | 70 | 20 | 0 | 10 |
| S-22 | 70 | 20 | 5 | 5 |
| S-23 | 70 | 20 | 10 | 0 |
| S-24 | 70 | 25 | 0 | 5 |
| S-25 | 70 | 25 | 5 | 0 |

This ratio was defined taking into account that, generally, in a fruit juice, the fruit/water ratio is approximately 35:65 (FÜGEL *et al.*, 2005) and in this study water was replaced by grape juice. Because for each beverage at least two fruit juices must be present, a total of 25 mixed juices were obtained. The prototypes were then bottled, pasteurised (105°C, 25 min) and stored at ambient temperature.

Three replicates for each of the 25 recipes are been prepared.

### Reagents

Folin-Ciocalteu reagent, sodium hydroxide, glucose, fructose, phosphoric acid, methanol, sulphuric acid, caesium chloride, tartaric, malic and citric acids were purchased from Sigma-Aldrich (Milano, Italy). Ultrapure water was obtained from a Milli-Q gradient A10 instrument (Millipore Corporation, Billerica, USA).

### Analyses

Density, extract, pH, sulphur dioxide, titrable acidity, total sugars, glucose, fructose, ashes and potassium of grape must were determined in accordance with the Commission Regulation (EEC) No. 2676/90 of 17 September 1990, while tartaric, malic and citric acids were determined by HPLC (CANE, 1990). The polyphenolic composition of the grape must and fruit juices (total polyphenols, anthocyanin and flavonoid contents) was determined by spectrophotometry (DI STEFANO *et al.*, 1989). The glucose, fructose, total sugars, ashes, titrable acidity, pH, tartaric acid, malic acid, citric acid and potassium of the fruit juices and beverages were determined in accordance with Italian Standard Methods (DM 03/02/1989). The fruit juice antioxidant capacity, expressed as Vitamin-C Equivalent Amount or VEAC Index, was determined according to KIM *et al.* (2002). The colour was measured using a Konica Minolta spectrophotometer CM-5 (Minolta Corp, Osaka, Japan) in the CIELab colour system with a D65 illuminant. The parameters measured were L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Each sample was evaluated in a 40-mL cuvette (1-cm thickness). All evaluations were performed in triplicate.

### Liking test

As reported by MAMMASSE and SCHLICH (2014), literature recommend a range from 50 to 100 consumers in hedonic tests and generally no replication are needed. Taking into account this and the limited quantity of samples, the liking test was executed once by recruiting 50 consumers (22 males and 28 females, aged 26-65 years).

They have received an invitation and voluntarily have participated to the tests. All tests were conducted individually, and social interaction was not permitted. The test was performed inside an air-conditioned meeting room with white light. The temperature was approximately 21 °C, and the relative humidity was approximately 50%. Tests were performed from 11 a.m. over 5 days. For each session, five experimental beverage samples (approximately 30 mL each) were presented in a completely randomised and balanced order. The samples were offered to the consumers in coded plastic cups. Natural bottled water was provided to each participant for palate cleansing. To decrease fatigue, there was a 5 minutes break between each sample. During each break, the consumers rinsed their mouths with water. All beverages were evaluated for specific parameters by consumers on a Just-about-right (JAR) structured scale, and then the consumers were asked to express the overall pleasantness of each product.

For JAR evaluation, consumers rated the samples on a 5-point JAR scale (1 = much too low, 2 = a little too low, 3 = just about right-JAR, 4 = a little too much, and 5 = much too much) for five sensory parameters: colour, odour, aroma, sweet taste and persistence in the mouth. For the overall pleasantness evaluation, a segment of known length (100 mm), limited to the extremes of two adjectives of opposite meaning (bipolar scale) was used. Consumers were asked to mark the line that corresponded to their degree of overall pleasantness. The data were collected on a paper card. According to PAGÈS *et al.* (2014), the 5 JAR variables were reduced to 3 for data evaluation: "not enough" (by grouping the "much too low" and "a little too low" responses), "JAR" and "too much" (by grouping the "much too much" and "a little too much" responses). This grouping of variables leads to simpler analyses, and it allows for obtaining more stable results because non-JAR categories are associated with higher frequencies (PAGÈS *et al.*, 2014).

### Statistical analysis

Compositional data and overall pleasantness were examined by one-way analysis of variance (ANOVA) with Tukey's test ($p \leq 0.05$) as a multiple range test with XLSTAT 2011 (Addinsoft SARL, California, USA) and then used for a Principal Component Analysis, also performed with XLSTAT 2011 (Addinsoft SARL, California, USA). The °Brix/acid ratio and the overall pleasantness were subjected to Pearson's test (r). The JAR data were subjected to a penalty analysis with XLSTAT-MX 2014.2.07 (Addinsoft SARL, California, USA).

## RESULTS AND DISCUSSION

### Compositional aspects

The compositions of grape and fruit juices used for beverage production are reported in Table 2, while the composition of the obtained beverages are reported in Tables 3 and 4.

As highlighted by MORALES-DE LA PENA *et al.* (2010), the overall quality of a fruit juice

Table 2 - Composition of grape and fruit juices used for beverages production. Data are expressed as mean ± SD.

| | Fruit juices | | | Grape juice | |
|---|---|---|---|---|---|
| | Pear | Peach | Apple | | |
| Glucose (g $L^{-1}$) | 19.08±0.2 | 39.78±0.2 | 20.41±0.2 | Glucose (g $L^{-1}$) | 86.94 ±0.2 |
| Fructose (g $L^{-1}$) | 75.43±0.4 | 41.59±0.5 | 61.94±0.2 | Fructose (g $L^{-1}$) | 94.82 ±0.09 |
| Ashes (g $Kg^{-1}$) | 2.6±0.03 | 4.3±0.02 | 2.7±0.02 | Ashes (g $L^{-1}$) | 3.4 ±0.1 |
| Potassium (mg $Kg^{-1}$) | 1720±0.2 | 3542±6 | 1540±0.4 | Potassium (mg $Kg^{-1}$) | 1223±5 |
| °Brix | 13.5±0.4 | 11.5±0.3 | 11±0.2 | °Brix | 19±0.3 |
| Total Acidity (g $L^{-1}$) | 4.3±0.2 | 4.85±0.3 | 4.13±0.1 | Total Acidity (g $L^{-1}$) | 6.26±0.04 |
| pH | 3.73±0.03 | 3.79±0.01 | 3.76±0.01 | pH | 3.36±0.05 |
| Tartaric acid (g $L^{-1}$) | 0.281±0.01 | 0.233±0.02 | 0.26±0.03 | Tartaric acid (g $L^{-1}$) | 2.73±0.02 |
| Malic acid (g $L^{-1}$) | 0.312±0.03 | 0.892±0.02 | 1.028±0.01 | Malic acid (g $L^{-1}$) | 2.35±0.03 |
| Citric acid (g $L^{-1}$) | 0.588±0.03 | 0.788±0.04 | nd | Citric acid (g $L^{-1}$) | 0.1 ±0.01 |
| Polyphenols (mg $Kg^{-1}$) | 126.7±5 | 81.9±4 | 96.5±4 | Polyphenols (mg $L^{-1}$) | 446 ±6 |
| | | | | Density (g $L^{-1}$) | 1.07715 ±0.0004 |
| | | | | Extract (g $L^{-1}$) | 206.6±0.4 |
| | | | | Free Sulphur Dioxide (mg $L^{-1}$) | nd |
| | | | | Total Sulphur Dioxide (mg $L^{-1}$) | 11.2±0.4 |
| | | | | Anthocyanins (mg $L^{-1}$) | 228 ±1.74 |

(nd – not determined).

is evaluated by a few parameters such as soluble solids, pH and acidity. The grape juice displayed a total soluble solids content of 19 °Brix with approximately 170 g $L^{-1}$ of sugars, while the peach, apple and pear juices exhibited 11.5, 11.0 and 13.5 °Brix, respectively. The mean value of °Brix for new beverages was approximately 15.3, with a significant reduction with respect to grape juice, approximately 19. The obtained value is similar to that of a fruit juice (GUNATHILAKE *et al.*, 2014) and ideal for the formulation of nutraceutical food beverages (SARAVANAN and ARADHYA, 2011a). The content of fructose in apple juice (approximately 62 g $L^{-1}$) was higher than that reported by WU *et al.* (2007) but lower than that reported by WILL *et al.* (2008) and MARKOWSKI *et al.* (2009). Additionally, the fructose content of pear juice (approximately 75 g $L^{-1}$) was higher than that reported by COLARIC *et al.* (2006). Acidity is one of the most important quality parameters for fruit juices (BHARDWAJ and PANDEY, 2011), as confirmed by AL BITTAR *et al.* (2013), who included this factor in the sensory analysis of an innovative grape juice enriched in polyphenols. Nevertheless, LIU *et al.* (2006) highlighted that: "high acidity has a negative influence on the palatability of table grapes, as well as the suitability for wines". The value of the total acidity, expressed as tartaric acid, of the grape juice used in this study (6.26 g $L^{-1}$) is comparable to that reported for juices made with different grape cultivars (MARSELLÉS-FONTANET *et al.*, 2013; LIU *et al.*, 2006; SOYER *et al.*, 2003). The main organic acid in grape is tartaric acid, which has a $pK_1$ of 3.04, followed by malic acid, which has a $pK_1$ of 3.40 (LIU *et al.*, 2006). The grape juice had a tartaric acid content of 2.73 g $L^{-1}$, similar to juice reported by LIU *et al.* (2006). The pH value also plays an important role in the preparation of beverages (BHARDWAJ and PANDEY, 2011). The blending process here studied is aimed to increase the pH value of grape must (3.36). Our obtained results indicated that the addition of fruit juice with pH values of 3.79 (peach), 3.76 (apple) and 3.73 (pear) increased the pH of grape juice so that it reached a mean value of 3.44 in the prepared beverages. In his research on the properties of fruit juices used for functional beverages, GUNATHILAKE *et al.* (2014) reported a pH of 3.60 for apple juice, while ANDRÉS *et al.* (2014) in their evaluation of the bioactive compounds in non-fermented beverages highlighted that the pH ranged between 3.20 and 4.01, in agreement with SAARELA *et al.* (2011). Typically, the pH values of fruit juices are below 4, or even 3, depending on the fruits used. The amount of organic acids in the fruit juices depended on the cultivar: apple displayed the highest amount of malic acid, with a content of 1.028 g $L^{-1}$, while pear juice had the highest citric acid content (0.588 g $L^{-1}$). AGUILAR-ROSAS *et al.* (2007) reported a malic acid content below 0.35 g $L^{-1}$ for the same cultivar, whereas BURON-MOLES *et al.* (2014) reported a malic acid content of 1.4 g $L^{-1}$. For beverages, the most abundant organic acid was malic acid, with a mean content of 2.84 g $L^{-1}$, while the mean tartaric acid amount in these samples was 2.39 g $L^{-1}$. For this compound, the concentration was similar among all of the beverages because the same quantity of grape must was used and because the quantity of tartaric acid is very low for fruit juice. The highest values were

Table 3 - Composition of samples obtained by mixing grape juice and fruit juices of pear, peach and apple and results of Anova with Tukey's test. Data are expressed as mean ± SD. For samples code see Table 1. Values in each column having different letters are significantly different at p<0.05.

| Sample code | Extract (g L-1) | °Brix | Glucose (g L-1) | Fructose (g L-1) | Acidity (g L-1) | pH | Organic acids (g L-1) | | | Ashes (g kg-1) | Potassium (mg kg-1) | °Brix/ acid ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | tartaric | malic | citric | | | |
| S-1 | 167.85±0.21 | 15.75±0.35 b | 66.19±0.01 def | 80.3±0.71 cd | 6.00±0.00 j | 3.44±0.01 fgh | 2.52±0.00 ab | 3.45±0.00 ab | 0.55±0.03 cdefg | 2.6±0.01 bc | 1243±1.68 bcdefg | 26.25 |
| S-2 | 177.90±0.00 | 17.00±0.00 a | 71.40±1.41 abcd | 85.10±1.56 bc | 6.15±0.00 h | 3.45±0.01 cdefg | 2.51±0.00 ab | 3.32±0.09 ab | 0.73±0.02 defg | 2.7±0.02 bc | 1298±4.15 abcdef | 27.64 |
| S-3 | 181.80±0.00 | 17.00±0.00 a | 75.53±0.62 ab | 87.44±0.55 ab | 6.49± 0.06 ef | 3.43±0.01ghi | 2.69±0.03 ab | 3.54±0.05 a | 0.94±0.04 abcde | 2.8±0.01 bc | 1385±9.72 abc | 26.19 |
| S-4 | 183.10±0.00 | 17.00±0.00 a | 76.18±0.81 a | 85.95±0.78 abc | 6.75±0.00 c | 3.45±0.00 defg | 2.76±0.34 a | 3.60±0.26 a | 1.32±0.30 ab | 3.1±0.01 abc | 1424±2.64 ab | 25.19 |
| S-5 | 181.45±0.21 | 17.20±0.00 a | 75.47±0.18 ab | 83.01±0.09 bc | 6.94±0.00 b | 3.44±0.01 fgh | 2.66±0.18 ab | 3.47±0.20 ab | 0.28±0.02 fg | 3.2±0.01 ab | 1464±2.51 a | 24.78 |
| S-6 | 176.25±0.21 | 16.80±0.00 a | 68.45±0.64 cde | 85.78±0.82 bc | 7.20±0.00 a | 3.43±0.00 ghi | 2.47±0.01ab | 3.22±0.02 abc | 0.18±0.00 g | 2.6±0.01 c | 1161±5.66 cdefg | 23.33 |
| S-7 | 174.30±0.00 | 16.80±0.00 a | 70.39±1.42 bcd | 85.02±1.56 bc | 6.45±0.00 f | 3.45±0.01 defg | 2.51±0.04 ab | 3.21±0.06 abc | 0.20±0.02 g | 2.7±0.01 bc | 1233±0.69 bcdefg | 26.05 |
| S-8 | 178.05±0.21 | 16.90±0.14 a | 73.62±3.69 abc | 87.18±2.48 ab | 6.60±0.00 d | 3.45±0.01 defg | 2.37±0.18 ab | 3.18±0.01 abcd | 0.22±0.03 g | 3.2±0.00 ab | 1275±0.65 abcdef | 25.61 |
| S-9 | 180.50±0.00 | 16.8±0.00 a | 73.33±1.82 abc a | 86.15±0.62 ab | 6.51±0.03 e | 3.43±0.01 ghi | 2.64±0.05 ab | 3.30±0.07 ab | 1.18±0.00 ab | 3.1±0.01 abc | 1304±0.16 abcdef | 25.81 |
| S-10 | 181.70±0.14 | 17.00±0.00 a | 75.70±0.82 ab | 86.54±0.41 ab | 6.64±0.00 d | 3.40±0.00 j | 2.47±0.13 ab | 3.22±0.01 abc | 0.27±0.03 fg | 3.2±0.03 ab | 1370±3.08 abcd | 25.60 |
| S-11 | 182.10±2.23 | 17.00±0.00 a | 76.33±1.14 a | 84.12±1.20 bc | 6.75±0.00 c | 3.40±0.00 j | 2.60±0.00 ab | 3.09±0.09 abcde | 0.21±0.00 g | 3.5±0.01 a | 1328±5.83 abcde | 25.19 |
| S-12 | 148.60±0.00 | 14.65±0.21 c | 51.46±0.10 g | 75.87±0.57 de | 5.55± 0.00 o | 3.39±0.00 j | 2.30±0.04 ab | 2.87±0.04 bcdef | 0.35±0.27 efg | 2.6±0.01 c | 1059±9.92 g | 26.40 |
| S-13 | 134.00±0.00 | 13.10±0.14 e | 45.21±0.16 hi | 68.31±0.29 fg | 5.70± 0.00 m | 3.48±0.01 abc | 2.21±0.04 ab | 2.60±0.06 cdefg | 0.79±0.14 bcdefg | 2.6±0.01 c | 1156±9.72 efg | 22.98 |
| S-14 | 132.90±0.00 | 13.00±0.00 e | 45.68±0.25 hi | 66.86±0.44 g | 5.63±0.00 n | 3.50±0.00 a | 2.38±0.09 ab | 2.65±0.08 cdefg | 0.93±0.07 abcde | 2.8±0.01 bc | 1157±1.59 efg | 23.09 |
| S-15 | 136.00±0.00 | 13.10±0.14 e | 48.33±0.66 ghi | 67.92±0.91 fg | 5.93±0.00 k | 3.48±0.01 abc | 2.33±0.04 ab | 2.62±0.07 cdefg | 1.14±0.06 abc | 3±0.00 abc | 1222±3.08 bcdefg | 22.09 |
| S-16 | 138.50±0.14 | 13.20±0.00 e | 50.76±0.04 gh | 68.92±1.02 fg | 6.00±0.00 j | 3.48±0.00 ab | 2.29±0.03 ab | 2.59±0.03 cdefg | 1.51±0.20 a | 3.1±0.01 abc | 1245±3.94 bcdefg | 22.00 |
| S-17 | 134.60±0.14 | 13.20±0.00 e | 44.51±0.35 i | 70.63±0.42 efg | 5.55±0.00 o | 3.46±0.00 bcdef | 2.27±0.02 ab | 2.66±0.13 defg | 0.72±0.03 bcdefg | 2.6±0.03 bc | 1118±1.57 efg | 23.78 |
| S-18 | 135.9±0.14 | 13.20±0.00 e | 45.69±0.09 hi | 69.90±0.37 fg | 5.55±0.00 o | 3.47±0.00 bcd | 2.19±0.02 ab | 2.38±0.01 fg | 0.85±0.04 bcdef | 2.6±0.03 bc | 1121±0.49 efg | 23.78 |
| S-19 | 138.75±0.21 | 13.30±0.14 e | 46.98±0.12 ghi | 69.41±0.31 fg | 6.08±0.00 i | 3.47±0.01 bcde | 2.11±0.12 ab | 2.25±0.13 g | 1.00±0.02 abc | 2.9±0.01 abc | 1171±3.66 defg | 21.88 |
| S-20 | 139.55±0.21 | 13.20±0.00 e | 47.265±0.32 ghi | 67.77±0.11 fg | 6.08±0.00 i | 3.46± 0.00 bcdef | 2.02±0.29 b | 2.08±0.36 g | 1.06±0.10 abc | 3±0.01 abc | 1201±3.64 cdefg | 21.71 |
| S-21 | 141.10± 0.00 | 13.80±0.00 d | 45.38±0.10 hi | 72.92±0.30 ef | 5.78±0.00 l | 3.44±0.00 efgh | 2.04±0.18 b | 2.08±0.18 g | 0.76±0.21 bcdefg | 2.7±0.03 bc | 1098±1.96 fg | 23.88 |
| S-22 | 168.20±0.00 | 15.80±0.00 b | 60.86±1.21 f | 82.54±1.05 bc | 6.15±0.00 h | 3.44±0.00 efgh | 2.33±0.04 ab | 2.51±0.09 efg | 0.72±0.04 bcdefg | 2.8±0.01 bc | 1122±10.78 efg | 25.69 |
| S-23 | 169.80±0.00 | 15.80±0.00 b | 62.97±0.71 ef | 82.58±0.52 bc | 6.3±0.00 g | 3.42±0.01 hij | 2.40±0.00 ab | 2.49±0.10 efg | 1.19±0.05 ab | 3±0.01 abc | 1242±2.39 bcdefg | 25.08 |
| S-24 | 172.25±0.21 | 16.10± 0.14 b | 62.64±0.49 f | 85.65±0.63 bc | 6.30±0.00 g | 3.44±0.00 efgh | 2.37±0.03 ab | 2.41±0.02 fg | 1.12±0.17 abc | 3±0.01 abc | 1195±0.99 cdefg | 25.56 |
| S-25 | 176.50±0.14 | 17.00±0.00 a | 70.34±4.38 bcd | 91.74±5.41 a | 6.49±0.00 ef | 3.41±0.01 ij | 2.32±0.08 ab | 2.35±0.06 fg | 0.21±0.05 g | 2.9±0.02 abc | 1251±8.68 abcdefg | 26.19 |

found for beverages S-3 and S-4, which contained higher quantities of apple juice. The Brix/acid ratio (Table 3) is an important parameter usually used to control fruit quality. In this study a positive correlation (r = 0.54, *p≤0.01.*) resulted between it and the overall pleasantness in accordance with JAYASENA AND CAMERON (2007). These authors reported that the °Brix/acid ratio compared with the °Brix alone demonstrated a higher degree of association with the consumer acceptability and it appeared a very useful maturity indicator. The peach juice exhibited the highest potassium content. This characteristic determined an increase in the content of this important component in the beverages containing high percentages of peach, e.g., sample S-5. The lowest value was determined for sample S-12, which was obtained without peach juice. The total polyphenols content ranged between 265.5 mg $L^{-1}$ for beverage S-5 and 407 mg $L^{-1}$ for beverage S-24, with a mean value of 359.30 mg $L^{-1}$. According to the total polyphenol contents of fruit juices, higher values were exhibited by beverages with high percentages of pear juice. The same beverage also displayed some of the highest values for the flavonoid content (627.5 mg $L^{-1}$ , the highest) and antioxidant capacity (585 mg $L^{-1}$ , the second highest one). For this parameter, the result for S-24 was similar to that of beverage S-1 (593 mg $L^{-1}$). The lowest value for the antioxidant capacity (464.41 mg $L^{-1}$) was displayed by beverage S-5. These results highlighted that the most interesting findings were obtained with a high quantity of apple or pear juice in the beverage, while a high content of peach juice led to a reduction of this parameter. The ANOVA and Tukey's test performed for each parameter of the beverages displayed high variability among all samples and strictly corre-

Table 4 - Polyphenol composition (PHEN - total polyphenols; TAI - anthocyanins; TFI - flavonoids; VCEAC - antioxidant capacity) and CIELab values of samples obtained by mixing grape juice and fruit juices of pear, peach and apple and results of ANOVA analysis with Tukey's test. Data are expressed as mean ± SD. For sample code see Table 1. Values in each column having different letters are significantly different at p<0.05.

| Sample code | PHEN (mg $L^{-1}$) | TAI (mg $L^{-1}$) | TFI (mg $L^{-1}$) | VCEAC (mg $L^{-1}$) | L*(D65) | a*(D65) | b*(D65) |
|---|---|---|---|---|---|---|---|
| S-1 | 384.5±4.95 cd | 84±1.41 abc | 540.5±44.55 cdefgh | 593.83±10.40 a | 68.14±2.86 ab | 32.18±0.46 | 11.20±0.43 |
| S-2 | 389.5±2.12 bcd | 84.5±2.12 abc | 478±4.24 ghijkl | 578.38±1.04 ab | 64.75±0 .01 b | 33.9±0.10 | 11.675±0.15 |
| S-3 | 366±1.41 efg | 82±2.82 abcd | 407.5±20.51 l | 526.17±4.16 abcd | 66.28±0.45 ab | 33.56±0.19 | 11.36±0.26 |
| S-4 | 362±0.7 gh | 82.5±1.06 abc | 497±4.95 fghijk | 468.09±20.28 cd | 66.63±0.19 ab | 32.72±0.51 | 10.52±0.27 |
| S-5 | 265.5±6.36 o | 80±0.00 abcde | 447±2.83 ijkl | 464.41±10.40 d | 66.65±1.98 ab | 34.84±1.72 | 11.23±0.30 |
| S-6 | 402.5±4.95 ab | 88.5± 0.71 a | 612.5±0.71 abc | 534.26±48.87 abcd | 64.89±0.25 b | 32.94±0.40 | 11.31±0.28 |
| S-7 | 405.5±3.54 a | 88±0.00 ab | 576±1.41 abcd | 476.91±7.28 cd | 64.13±0.93 b | 32.75±0.07 | 10.89±0.01 |
| S-8 | 385.5±2.12 cd | 80.5±0.71 abcde | 546.5±7.78 bcdefg | 492.35±47.83 bcd | 63.85±1.52 b | 33.52±2.33 | 10.97±1.19 |
| S-9 | 380.5±2.12 de | 81.5±0.71 abcd | 545±5.66 cdefg | 530.59±29.12 abcd | 65.63±1.25 ab | 33.85±0.76 | 11.46±0.59 |
| S-10 | 362.5±2.12 fgh | 80.5±0.71 abcde | 517±1.41 defghi | 480.59±10.40 cd | 66.21±3.21 ab | 34.4±2.77 | 11.25±1.00 |
| S-11 | 338.5±4.95 ijk | 79.5±0.71 abcde | 490.5±.71 fghijk | 537.94±18.72 abcd | 67.96±2.23 ab | 33.8±1.33 | 10.9±0.72 |
| S-12 | 377±1.41 def | 82±0.00 abcd | 581±1.41 abcd | 577.65±0.00 ab | 66.62±0.40 ab | 33.49±1.94 | 9.45±0 .47 |
| S-13 | 334±0.00 jkl | 67.5±0.71 gh | 515.5±4.95 defghi | 551.18±0.00 abcd | 67.5±2.21 ab | 31.66±0.46 | 9.81±0.01 |
| S-14 | 325±4.24 klm | 63.5±2.12 h | 493.5±4.95 fghijk | 510±2.08 abcd | 68.51±1.62 ab | 32.66±1.77 | 10.04±0.04 |
| S-15 | 314.5±0.71 mn | 68.5±2.12 gh | 430±15.56 kl | 506.32±11.44 abcd | 70.09±0.14 ab | 30.79±1.81 | 9.77±0.95 |
| S-16 | 309.5±0.71 n | 65.5±0 .71 gh | 436±9.90 jkl | 510.74±15.60 abcd | 74.03±4.72 a | 31.21±1.16 | 9.26±1.27 |
| S-17 | 350±4.24 hi | 72±1.41 efgh | 552.5±6.36 bcdef | 583.53±12.48 a | 66.6±0.62 ab | 32.86±1.14 | 11.09±0.09 |
| S-18 | 342±1.42 ij | 69.5±2.12 fgh | 514±2.83 defghi | 551.17±33.28 abcd | 68±2.18 ab | 33.46±1.28 | 10.93±0.60 |
| S-19 | 333±1.14 jkl | 67±0.00 gh | 503±0.00 efghij | 540.88±6.24 abcd | 69.48±1.60 ab | 32.71±1.05 | 10.76±0.69 |
| S-20 | 321±1.41 lmn | 64.5±2.12 gh | 469±8.49 hijkl | 551.18±29.12 abcd | 67.82±2.71 ab | 33.63±2.10 | 9.95±0.09 |
| S-21 | 365±4.24 fg | 68±0.00 gh | 571±1.41 abcde | 546.76±12.48 abcd | 67.49±0.49 ab | 32.6±1.43 | 10.56±1.41 |
| S-22 | 395.5±0.71 abc | 80±7.07 abcde | 618±49.5 ab | 576.91±7.28 ab | 63.95±1.63 b | 34.42±0.86 | 11.88±0.30 |
| S-23 | 376±2.83 defg | 73±1.41 defg | 556±0.00 abcdef | 548.24±0.00 abcd | 70.73±5.76 ab | 32.09±1.22 | 10.05±1.86 |
| S-24 | 407±7.07 a | 78±1.41 cdef | 627.5±4.95 a | 585±37.43 a | 65.57±1.23 ab | 32.27±0.23 | 11.68±0.23 |
| S-25 | 390.5±7.78 bcd | 79±5.66 bcde | 576±46.67 abcd | 556.32±1.04 abcd | 66.51±0.28 ab | 32.82±0.91 | 10.89±0.59 |

lated with the composition of each single fruit juice and the different percentages used for beverage production. In fact, there were no differences between the beverages for the CIELab parameters a* and b* only, and this is due to the high grape must percentage used.

**Sensory aspects**

Concerning the overall pleasantness, the ANOVA highlighted significant differences among the 25 experimental beverages (Table 5).

Even if the content of grape juice was kept constant in all of the beverages at 70%, the different percentages of other fruit juices can influence the acceptability. The most appreciated samples (S-22, S-24 and S-23) had the highest pear juice percentages, while the least appreciated (S-14, S-12, S-1 and S-16) had the lowest pear juice concentrations. The least appreciated was beverage S-14, which was obtained with a mix of apple, pear and peach juices at the same percentage (10%). Penalty analysis was used because with this test it is possible to identify the sensory attributes that have the largest influence on consumer liking and provides directions for product reformulation (ARES *et al.*, 2014) and also allows one to determine if a specific product attribute is "just about right" (TAYLOR, 2013). Penalty analysis combines JAR variables and overall liking tests to find correlations between a decrease in consumer acceptance and attributes not at the JAR level. This analysis, based on multiple comparisons, is aimed to identify and determine if the rankings on the JAR scale are related to significantly different results in the liking scores for each sensory attribute studied on the JAR scale. This can be achieved by evaluating the mean decrease in overall liking versus percentage of not-JAR variables (i.e., the low percentage of not-JAR evaluation determines a low mean decrease in overall liking). When some not-JAR categories receive at least 20% (Pareto principle) responses for an attribute, this becomes a candidate for penalty analysis. Penalty analysis uses the 20% cut-off theory on the percentage of not-JAR consumers based on the Pareto principle (i.e., the Pareto principle recognises that "80% of effects occur from 20% of causes" or the 80-20 rule) and signifies several common occurrences in everyday phenomena. Therefore, the 20% cut-off is used as a general rule for penalty analysis (NARAYANAN *et al.*, 2014). In Fig. 1 are reported the JAR scores for each parameter used in the beverage evaluation.

The colour was judged "just right" by 50% of the consumers, odour by 37%, aroma by 38% and persistence in the mouth by 39%. In general, the "JAR" value was chosen by a higher number of assessors: the higher frequency was highlighted by the "a little too low" val-

Table 5 - Mean values of overall pleasantness and results of ANOVA and Tuckey's test. Data are expressed as the mean ±SD. Values with different letters are significantly different at p<0.05.

| Sample code | Fruit juices | | | Overall pleasantness | Tuckey test (p< 0.05) |
|---|---|---|---|---|---|
| | pear (%) | peach (%) | apple (%) | | |
| S-22 | 20 | 5 | 5 | 56.98 | a |
| S-24 | 25 | 0 | 5 | 55.3 | ab |
| S-23 | 20 | 10 | 0 | 55.14 | ab |
| S-8 | 5 | 10 | 15 | 54.18 | abc |
| S-9 | 5 | 15 | 10 | 54.08 | abc |
| S-2 | 0 | 10 | 20 | 54.04 | abc |
| S-25 | 25 | 5 | 0 | 53.07 | abcd |
| S-5 | 0 | 25 | 5 | 53.36 | abcde |
| S-11 | 5 | 25 | 0 | 52.36 | abcdef |
| S-3 | 0 | 15 | 15 | 50.82 | abcdefg |
| S-4 | 0 | 20 | 10 | 50.56 | abcdefg |
| S-6 | 5 | 0 | 25 | 50.54 | abcdefg |
| S-7 | 5 | 5 | 20 | 49.72 | abcdefg |
| S-10 | 5 | 20 | 5 | 48.9 | abcdefg |
| S-21 | 20 | 0 | 10 | 48.38 | abcdefg |
| S-15 | 10 | 15 | 5 | 46.36 | abcdefgh |
| S-20 | 15 | 15 | 0 | 45.7 | bcdefgh |
| S-17 | 15 | 0 | 15 | 43.8 | cdefgh |
| S-18 | 15 | 5 | 10 | 42.9 | defgh |
| S-19 | 15 | 10 | 5 | 42.68 | efgh |
| S-13 | 10 | 5 | 15 | 42.6 | efgh |
| S-16 | 10 | 20 | 0 | 42.46 | fgh |
| S-1 | 0 | 5 | 25 | 41.96 | fgh |
| S-12 | 10 | 0 | 20 | 40.86 | gh |
| S-14 | 10 | 10 | 10 | 36.36 | h |

ue for only the odour. Fig. 1 also demonstrates that the "much too much" and "much too low", although they may affect the overall pleasantness, do not weigh significantly on it because of their low frequency in the responses of consumers. The variables can then be grouped into two main groups with "a little too much" or "a little too low". The first group corresponds to "much too much", while the second corresponds to "not enough" for the parameters of

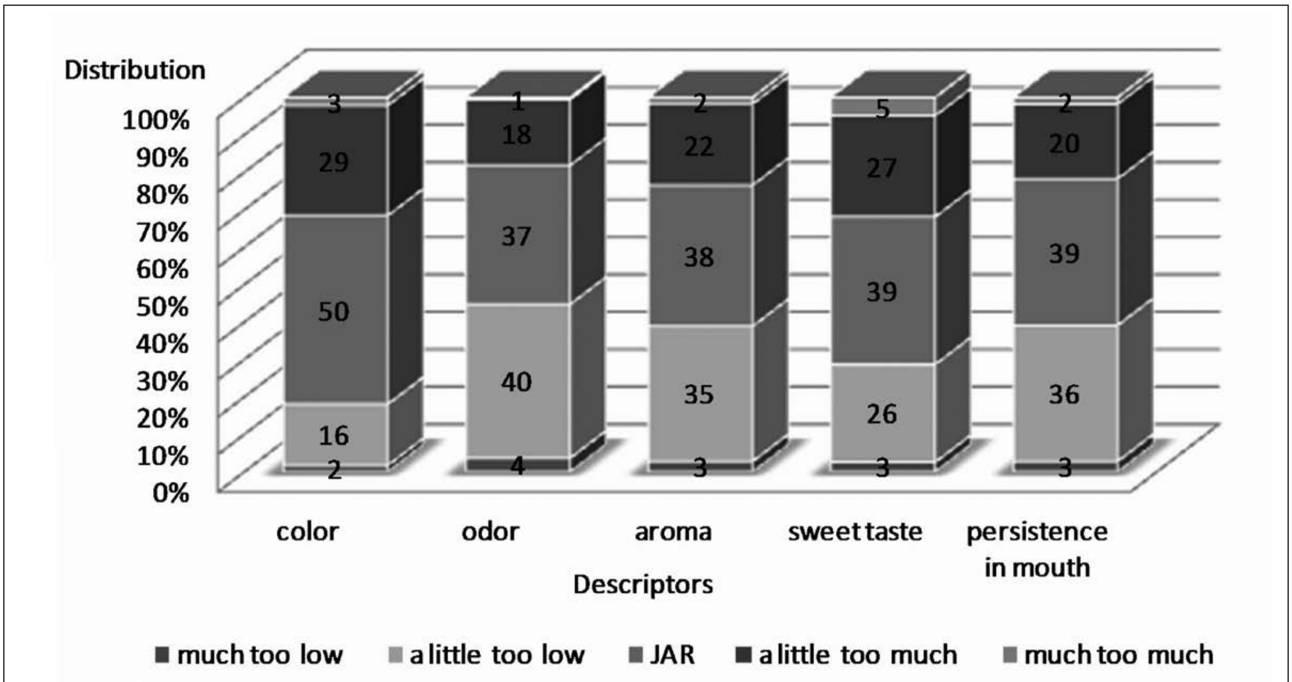


Fig. 1 - Distribution of JAR scores for each sensory attribute evaluated.

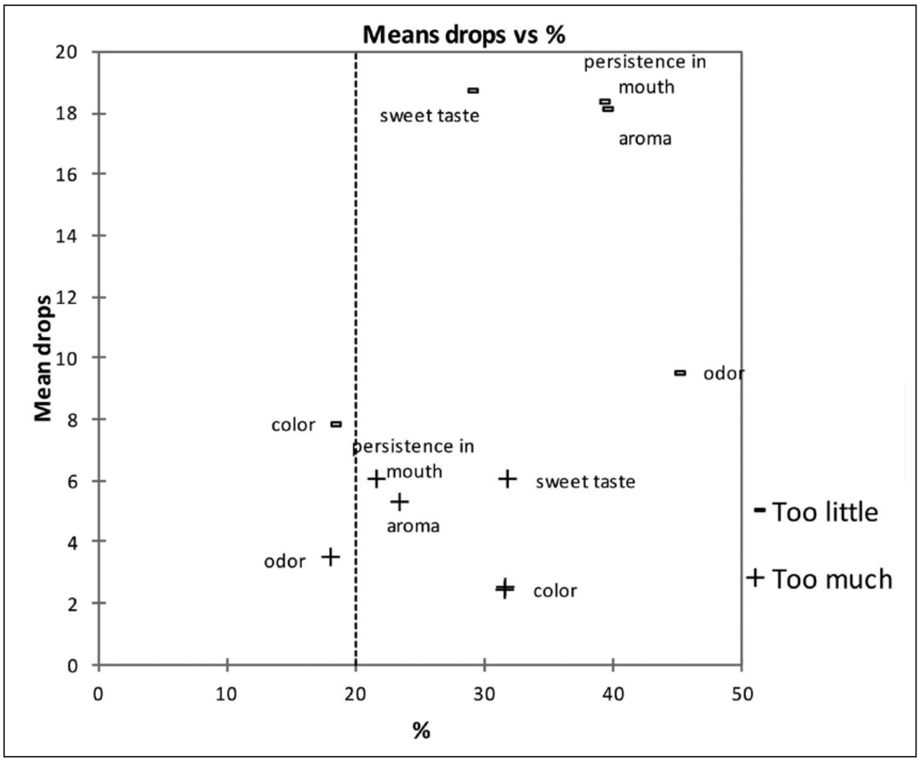


Fig. 2 - Penalty analysis from JAR data. Not-JAR data with a frequency <20% of total responses are not considered significant.

colour, aroma, sweet taste and persistence in the mouth. In Fig. 2 are displayed the distribution of frequency and then their effect on the mean drop in overall pleasantness.

Sweet taste, aroma and persistence in mouth exhibited a higher effect on the overall pleasantness if classified as "not enough". Also important for determining the overall pleasantness was the odour, if classified as "not enough". When the sensory parameters were classified as "too much", they had less impact on the overall pleasantness. A principal component analysis was also performed to highlight the correlation between chemical-physical parameters and overall pleasantness. The first two components explained 72.82% of the variance (Fig. 3).

The first component explained 50.74% of the variance and was mainly correlated with the to-

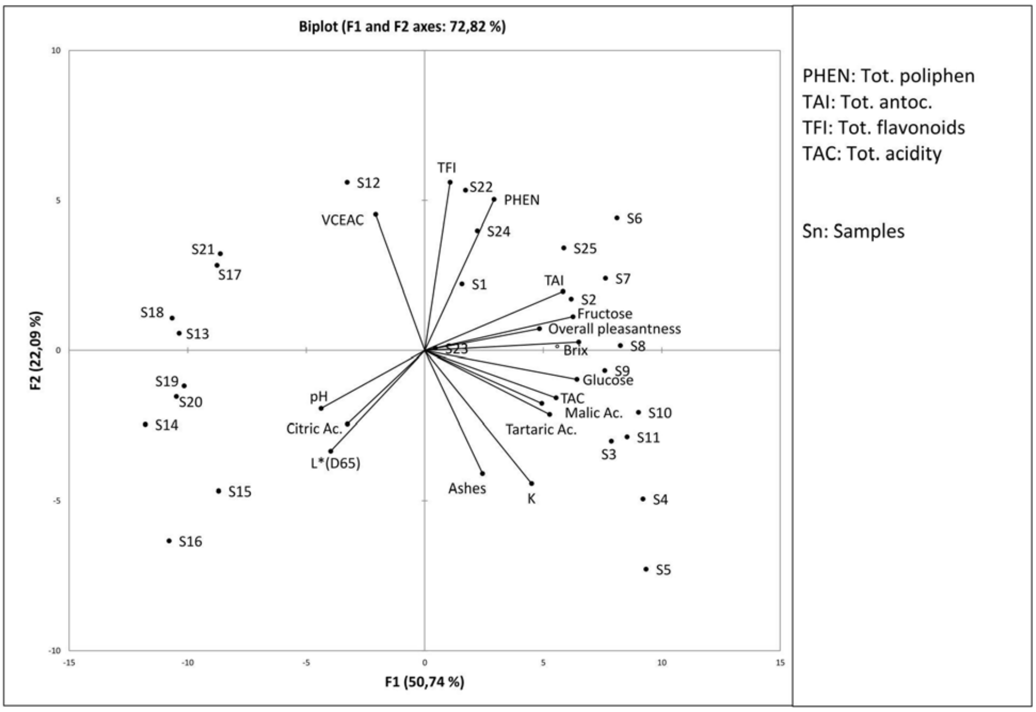


Fig. 3 - Distribution on plane defined by the first two components of chemical-physical parameters, overall pleasantness and beverage samples.

tal soluble solids, glucose and fructose contents, which corresponded to 12.1, 11.8 and 11.2%, respectively, of the total variance explained by this axis. The second component that explained 22.08% of the total variance is associated with the flavonoids, total polyphenols and antioxidant capacity, accounting for 20.7, 16.7 and 13.5%, respectively, of the total variance explained by this axis. The overall pleasantness was positively correlated with the contents of the total soluble solids and fructose and negatively correlated with the pH, citric acid content and L*. Because the overall pleasantness is located in the upper right graph quadrant, all of the beverages placed in the same quadrant are the most appreciated. In particular, the highest appreciation was found for the S-22, S-24 and S-23 samples, as also demonstrated by Table 5. The less appreciated samples are on the lower left side of the graph. They can be grouped into two groups: S-16, S-15, S-14, S-20 and S-19 in the lower left quadrant of the PCA graph and S-13, S-18, S-17, S-21 and S-12 in the upper left quadrant. The first group exhibited a more transparent colour, with a high value for L* and higher pH and citric acid contents. The second group demonstrated a low overall pleasantness but a high antioxidant capacity. In this group, it must be highlighted that beverage S-14, with the same percentages of fruit juice (10-10-10), had the lowest appreciation and the highest pH.

## CONCLUSIONS

One of the first internationally accepted descriptions of functional food has been provided by DIPLOCK *et al.* (1999) according to which: "a food product can be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism...". Taking this into account, and also of the scientific evidence regarding the benefits of the products based on grapes to human health, the results obtained in this study have shown that these experimental fruit juices have functional characteristics. Additionally, as reported by BHARDWAJ and PANDEY (2011), it may be concluded that the formulation of mixed beverages can satisfy consumer tastes and preferences. In particular, the overall pleasantness results indicate a tendency of consumers to prefer samples with the highest percentage of pear juice, followed by samples containing mixtures of peach and apple juices.


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