

Effect of the Storage Time and Packaging Material on the Antioxidant Capacity and Phenols Content of Organic Grape Juice Stabilized by High Hydrostatic Pressure

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Grape (*Vitis vinifera* L.) is widely used in juice industries and it contains a variety of polyphenols with antioxidant and anti-inflammatory properties. The effect of packaging materials and storage time on the phenol content and antioxidant stability of grape juices made with a Merlot variety was studied. In order to preserve sensory and nutritional values, the juice was stabilized with a high hydrostatic pressure (HHP) and a polylactic acid (PLA) bottle was tested as a substitute for the polyethylene terephthalate (PET) packaging. Except for *o*-diphenols, the spectrophotometric assays of the different phenol classes showed higher values in juice after HHP treatment. Overall, a loss of most of the phenols was tested in PLA bottle samples at four months of shelf-life. On the contrary, juices in PET bottle did not show significant changes until the end of storage. A comparable trend was observed also for the antioxidant activity and the individual phenols analyzed by a liquid chromatography-diode array detection-mass spectrometry (HPLC-DAD-MS), which showed anthocyanins as principal class of phenols, followed by flavonols and hydroxycinnamoyl tartrates (HCTs). These results on grape juice stability could be interesting not only for the juice production but also for the development of the best packaging material.

1. Introduction

It is common knowledge that the consumption of fruit and vegetable is associated with numerous health benefits, mainly due to their high content in polyphenols. Grape (*Vitis vinifera* L.) has proven to be among the fruits with the highest content of these health-protecting compounds that are principally flavonoids such as anthocyanidins, flavonols, flavan-3-ols, and proanthocyanidins, and nonflavonoids such as hydroxycinnamoyl tartrates (HCTs) (Ferrandino et al., 2012).

Several studies report how many polyphenols found in grape and its products (i.e. flavan-3-ols, quercetin and anthocyanins) have an important role in the development of color and organoleptic characteristics, in addition to preventing various oxidative and inflammation diseases (Day et al., 1997; Singletary et al., 2003).

Emerging evidence suggests that fruit juices may be a good alternative because they are a natural source of phytochemicals with beneficial effects comparable to the fresh fruit (Wootton-Beard and Ryan, 2011). In order to satisfy the growing consumption of grape juice as a source of antioxidants, it is important to understand the influence of the process and storage conditions on the total phenolics content in the final product.

Shelf-life and safety of juices have been traditionally achieved by thermal processing having the disadvantage to degrade many phenolic compounds and, consequently, decrease the product quality. Therefore, some non-thermal pasteurization processes were proposed, including high hydrostatic pressure (HHP), a cold pasteurization, which can assure fruit juice stability for an extended period, maintaining the original juice color, flavour, aromas and nutritional and functional characteristics (Bevilacqua et al., 2018).

The packaging systems have also an important role to protect foods and increase their shelf-life. One of the materials traditionally used in beverages is plastic, even if there is a growing consumers' attention for sustainable materials. At present, one of the trends in food packaging is the use of bio-polymers that are "eco-

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friendly," biodegradable, and made from natural resources such as polylactic acid (PLA). PLA is a sustainable alternative to petrochemical-derived products, that can be produced by chemical synthesis or fermentation (Pretula et al., 2016). PLA is the polymer showing the highest potential for a commercial major-scale production of renewable packaging materials and it has a good water vapour barrier and relatively low gas transmittance.

Within this context, the main aim of this research was to investigate the changes of the levels of different polyphenol classes in grape juices, stabilized with the HHP treatment, during six months of shelf-life at 4 °C. Besides, the storage stability of the fruit juice was evaluated comparing two different packaging materials: PET and PLA.

2. Materials and Methods

2.1 Plant materials

Grapes (*Vitis vinifera* L.) from Merlot variety were collected from the organic vineyard of the "Cascina Belmonte" company, located in Muscoline (Brescia, Italy). Grapes used for juice production were manually harvested at the stage of technical maturity and processed through an industrial-scale technological process.

2.2 Grape juice processing, packaging and storage

After harvesting, grapes were washed and dried and the grape juice was produced without the addition of sugars, flavourings or preservatives. Juice was obtained with destemming and maceration at 10 °C for 48 h, followed by pressing and filtering. After that, juice was collected in 250 mL-sterilized plastic bottles (PET and PLA) and subjected to the HHP at 600 MPa for 3 minutes. After cold pasteurization, the grape juice bottles were immediately refrigerated at 4 °C in order to assure their stability for their commercial shelf-life.

The PET bottles were produced by injection stretch blow molding (ISBM) with industrial machines. All process parameters were optimized to ensure good crystallinity and wall thickness distribution. Neck to cap coupling geometry was also optimized. Prototype PLA narrow-neck bottles were also produced by ISBM using process parameters adjusted to PLA. Hence, several PLA bottle features, especially neck conformation, had not been fully optimized. Typical oxygen transmission rate (OTR) values are reported in the range 3.0-6.0 cc-mil/100 in² day atm (20 °C, dry) for PET and in the range 38-42 for PLA (Natureworks, 2018). PLA has a lower barrier to oxygen so that expected oxygen intake for juice packed in PLA bottle may be at least 6-10 times higher than juice in PET (at similar thickness).

Samples for analyses were taken on day 0 (T0), after 2 (T2), 4 (T4) and 6 months (T6) of refrigerate storage. Additionally, one sample was frozen before HHP and analyzed as untreated grape juice (control, T00).

2.3 Samples preparation

For a better storing, all the grape juices collected from the company in their original bottles were sampled in 50 mL plastic tubes and kept at -42 °C. Before analyses, juices were placed one night in refrigerator (+4 °C) and then they were centrifuged at 4000 rpm for 10 minutes. Each juice sample was analyzed in triplicate.

2.4 Spectrophotometric determination of phenolic compounds

The spectrophotometric assays were performed on a Shimadzu UV-1800 spectrophotometer. Juices were diluted in ultrapure water when necessary and analyzed without any extraction. The phenol index (PI), hydroxycinnamic acid index (HI), flavonol index (FI) and color index (Col) were obtained by the juice absorbance at 280, 320, 350 and 520 nm, respectively, using quartz cuvettes and room temperature (25 °C) (Bonoli et al., 2004). Gallic acid (1-25 µg/mL), ferulic acid (0.5-25 µg/mL), galangin (0.5-20 µg/mL) and delphinidin chloride (1-30 µg/mL) calibration curves were plotted to assess the PI, HI, FI and Col, respectively. The *o*-diphenolic compound index (ODI) was measured at 370 nm using quartz cuvettes (Danesi et al., 2013) and assessed with gallic acid calibration curve (1-25 µg/mL). Flavanols (Cal) were determined at 640 nm, using 4-(dimethylamino)-cinnamaldehyde (DAC) and glass cuvettes (Zironi et al., 1992). A catechin calibration curve from 0.5 to 20 ppm was used for quantification.

2.5 Antioxidant activity: ABTS assay

The antioxidant activity of the juices was evaluated by an *in vitro* protocol based on the inactivation of the stable synthetic radical ABTS^{•+} (Re et al., 1999). Data were expressed as mmol of Trolox equivalents L⁻¹.

2.6 Determination of phenolic compounds by HPLC-DAD-MS

An Agilent HP 1100 liquid chromatograph coupled with diode array and mass spectrometer detectors was used to determine the relative amount of the individual polyphenols in samples. According to Gómez-

Caravaca et al. (2013), a Poroshell 120 SB-C18 (3.0 × 100 mm, 2.7 µm) column and two different gradient elution programs depending on the phenolic classes were used for separation. Anthocyanins were analyzed without any extraction and juices were filtered with 0.2 µm RC filters before the injection. The chromatograms were registered at 520 nm and cyanidin-chloride (0.5-125 µg/L) was used as calibration curve. An electrospray ionization (ESI) interface in positive ion mode was used for MS analysis. In order to get a best HPLC analysis of other phenolic compounds, juices were freeze dried (250 mg) and suspended in 0.5 mL of distilled water. The extracts were vortexed, sonicated at 40 °C for 30 minutes, centrifuged at 15000 × g at 5 °C for 10 minutes and the supernatant filtered (0.2 µm RC filter). The chromatograms were registered at 280 and 330 nm. The calibration curves of gallic acid and catechin at 280 nm, and rutin and chlorogenic acid at 330 nm were arranged from 0.5 to 250 µg/mL. An ESI interface in negative ion mode was used for MS analysis.

2.7 Statistical analysis

Reported results are the averages of three extraction replications. One-way ANOVA (analysis of variance) followed by Tukey's HSD *post hoc* comparison test and Pearson's linear correlations, both at $p < 0.05$ level, were evaluated using Statistica 8.0 software (2007, StatSoft, Tulsa, OK, USA).

3. Results and Discussions

3.1 Spectrophotometric analysis of the phenol classes and the antioxidant activity in grape juices

The spectrophotometric investigation was carried out in order to evaluate the changes in antioxidants in grape juices after HHP treatment and during their storage in two different packaging materials (Table 1).

Table 1: Spectrophotometric indices of grape juice samples (mean values) during storage in PLA and PET bottles. (PI - phenol index, HI - hydroxycinnamic acid index, FI - flavonol index, Col - color index, ODI - o-diphenolic compound index, Cal – flavonols and ABTS - stable synthetic radical)

Packaging materials	Storage time	PI	HI	FI	Col	ODI	Cal	ABTS
		µg gallic acid/mL	µg ferulic acid/mL	µg galangin/mL	µg delphinidin chloride/mL	µg gallic acid/mL	µg (+)-catechin/mL	µmol Trolox equivalents/mL
Control [†]	00	175.69 ^e	56.62 ^d	74.96 ^e	102.32 ^{b,c}	1332.73 ^a	21.05 ^d	1.52 ^{b,c}
PLA	0	241.15 ^c	76.77 ^{a,b}	90.71 ^{b,c}	106.55 ^b	1120.61 ^b	29.17 ^b	2.00 ^{a,b}
	2	233.23 ^d	74.08 ^b	84.29 ^d	102.44 ^{b,c}	1029.70 ^b	28.15 ^{b,c}	1.94 ^{a,b}
	4	129.29 ^f	40.91 ^f	45.27 ^g	59.44 ^e	490.61 ^d	12.39 ^e	1.29 ^c
	6	176.73 ^e	53.73 ^e	52.17 ^f	79.24 ^d	405.76 ^d	5.57 ^g	1.33 ^c
PET	0	262.27 ^a	79.13 ^a	95.92 ^a	116.83 ^a	1066.06 ^b	32.24 ^a	1.99 ^{a,b}
	2	257.52 ^{a,b}	78.88 ^a	94.36 ^{a,b}	118.59 ^a	1060.00 ^b	29.12 ^b	1.93 ^{a,b}
	4	253.03 ^b	76.77 ^{a,b}	89.67 ^c	118.30 ^a	654.24 ^c	27.15 ^c	2.05 ^a
	6	229.66 ^d	66.51 ^c	71.44 ^e	98.03 ^c	451.21 ^d	9.93 ^f	1.79 ^{a,b,c}

[†] unprocessed sample

Different letter in the same column indicate significant differences ($p < 0.05$).

The PI represents the determination of the total phenol content of the juices, since phenols exhibited an absorption maximum at 280 nm. In accordance with literature (Inada et al., 2018), the fresh juice gave lower PI than HHP processed samples. The increase of about the 30% of PI after HHP could be related to an increase in solubilization of some antioxidant components into the juice. The samples with PLA packaging showed lower PI (-10%) than juice in PET bottles, with a significant decrease during shelf-life. A total phenol reduction was tested for PET samples only at six months of storage. The absorbances for HI, as well as for FI and for anthocyanins (Col) showed the same trend reported for PI. For all these indices, the HHP juices with PET packaging showed higher values compared to the control and the PLA counterparts. Besides, these last juices had a significant decrease during storage, whereas samples in PET bottles showed a strong decrease only at T6. Contrary to the other indices, the control sample presented the highest ODI (1332.73 µg/mL) and the packaging material did not affect the *o*-diphenols content in HHP juices, except for T4. Otherwise, the storage time influenced the ODI after T4 with a significant decrease in all samples. These results agree with literature because *o*-diphenols are known to be the most readily oxidized wine constituents. Diphenols improve radical stability by forming an intra-molecular hydrogen bond between the hydrogen of their hydroxyl group and their phenoxy semi quinone radical (Oliveira et al., 2011). The index of catechins (Cal), ranging

from 12.39 to 32.24 $\mu\text{g/mL}$, showed the lowest values compared to the other phenol classes. In this case, samples in PET bottles showed a significant decrease during shelf-life, even if with higher contents than PLA samples. Besides, the unprocessed sample had a lower content than the HHP juices. For all spectrophotometric indices, the juice packaged in PLA bottles at the fourth month of storage presented the lowest phenols content. A small increase was found in PLA packed juice at T6, probably due to interfering compounds, produced or modified during shelf-life, which could affect the spectrophotometric data. The antioxidant activities of juices, assessed by the ABTS method, showed values from 1.29 to 2.09 $\mu\text{mol Trolox equivalents/mL}$ (Table 1). As already reported in literature (Bevilacqua et al., 2018), the HPP treatments do not cause significant changes on antioxidant activity in juices, indeed control samples did not show statistical different values compared to HHP samples. The juice in PLA bottles recorded the lowest ABTS values at T4 and T6. As reported before, the HHP increased the free phenolic amount, whereas PLA containers have a lower barrier to oxygen, causing a loss of some phenolic compounds for oxidation. Positive Pearson's linear correlations between ABTS and the UV spectrophotometric indices were found. PI, HI, FI and Col showed high correlation coefficients (0.917, 0.932, 0.903, and 0.847, respectively). The correlation coefficients found with ODI and Cal was lower if compared with the others (0.785 and 0.414), nevertheless the significance was high for Cal (p -value <0.0001) and under p -value <0.05 for ODI.

3.2 Anthocyanins and other phenolic compounds content in grape juice samples by HPLC–DAD–MS

Seven anthocyanin-glucosides (three of them acylated) were identified in grape juices (Table 2). In accordance with Genova et al. (2012), the main anthocyanin was malvidin 3-glucoside, followed by peonidin 3-glucoside and malvidin 3-O (6-O-acetyl) glucoside. As for spectrophotometric assay, the control sample reported significant lower amounts of anthocyanins compared to HHP juices. For the first two months of shelf-life, packaging material did not affect the content of these phenols, whereas at T4, total anthocyanins, as well as the individual compounds, decreased significantly in juice with PLA bottles. The same loss was detected in PET packaging samples at T6. Cyanidin 3-O (6-O-acetyl) glucoside was the only one with an increase in juices during shelf-life, even if its concentration does not affect the trend of the total anthocyanins content.

Table 2: Anthocyanins content in grape juice samples during storage in PLA and PET bottles.

Packaging materials	Storage time	A1	A2	A3	A4	A5	A6	A7	Total
Control ¹	00	3.20 ^{b,c}	3.22 ^{b,c}	5.89 ^{b,c}	15.47 ^{b,c}	2.19 ^c	2.48 ^{b,c}	5.45 ^{b,c}	37.89 ^{b,c}
PLA	0	3.72 ^{a,b}	3.62 ^{a,b}	7.08 ^{a,b,c}	20.26 ^{a,b,c}	2.29 ^{b,c}	2.74 ^{a,b}	6.77 ^{a,b}	46.48 ^{a,b,c}
	2	3.83 ^{a,b}	3.63 ^{a,b}	7.32 ^{a,b,c}	20.81 ^{a,b}	2.36 ^{a,b}	2.70 ^{a,b}	6.29 ^{a,b}	46.94 ^{a,b}
	4	2.73 ^c	2.69 ^{c,d}	4.07 ^d	8.99 ^d	2.30 ^{b,c}	2.22 ^c	3.36 ^{d,e}	26.36 ^d
	6	2.72 ^c	2.67 ^d	4.00 ^d	8.65 ^d	2.38 ^{a,b}	2.20 ^c	3.17 ^e	25.78 ^d
PET	0	3.85 ^a	3.83 ^a	7.76 ^a	22.54 ^a	2.28 ^{b,c}	2.86 ^a	7.41 ^a	50.52 ^a
	2	3.66 ^{a,b}	3.69 ^{a,b}	7.43 ^{a,b}	20.16 ^{a,b,c}	2.34 ^b	2.72 ^{a,b}	6.14 ^{a,b}	46.14 ^{a,b,c}
	4	3.41 ^{a,b}	3.41 ^{a,b}	6.28 ^{a,b,c}	16.72 ^{a,b,c}	2.41 ^{a,b}	2.51 ^{a,b,c}	5.12 ^{b,c,d}	39.87 ^{b,c}
	6	3.21 ^{b,c}	3.22 ^{b,c}	5.62 ^{c,d}	14.15 ^{a,d}	2.49 ^a	2.37 ^{b,c}	4.17 ^{c,d,e}	35.24 ^{c,d}

¹ sample unprocessed

Different letter in the same column indicate significant differences ($p < 0.05$). Values are expressed as averages ($n=3$). A1: cyanidin 3-O-glucoside; A2: petunidin 3-O-glucoside; A3: peonidin 3-O-glucoside; A4: malvidin 3-O-glucoside; A5: cyanidin 3-O (6-O-acetyl) glucoside; A6: peonidin 3-O (6-O-acetyl) glucoside; A7: malvidin 3-O (6-O-acetyl) glucoside. Expressed as mg cyanidin-chloride/L.

Compared to the anthocyanins, other phenols were present in considerably lower amounts and differences in their contents were not as pronounced as described for the anthocyanins. In agreement with Cantos et al. (2002), quercetin 3-O-glucuronide and quercetin 3-O-glycoside were the main flavonols in all samples (Table 3). Despite the previous analyses, control sample had a similar concentration than the HHP stabilized juices and no differences were mostly found in PET bottle samples during shelf-life. On the contrary, PLA counterpart presented a significant lower content at T6, being around the 50% of the same samples at T2 and T4 and the 35% of the T0 one, for the total flavonol amount.

Procyanidin B1, catechin and epigallocatechin gallate were found as flavan-3-ols in all juices (Table 4). Despite some few changes for the individual compounds during shelf-life, the absence of significant differences among samples was found for the total content, with respect to both storage time and packaging materials. As for anthocyanins, flavonols showed a strong decrease during shelf-life because of their antioxidant activity. In fact, quercetin is one of the most effective antioxidant flavonoids (Cantos et al., 2002).

This is further demonstrated by the positive correlation between ABTS and total flavonol contents ($r^2 = 0.556$, $p < 0.05$), as already seen also for their spectrophotometric index (FI). No significant correlation was found between ABTS and the other phenol classes, whereas a high positive correlation was found for anthocyanins ($r^2 = 0.813$, $p < 0.0001$), probably due also to their significant higher content in juice samples compared to the other phenols.

Table 3: Flavonols content in grape juice samples (mean values) during storage in PLA and PET bottles. (F1: myricetin 3-O-glucoside; F2: quercetin 3-O-glycoside; F3: quercetin 3-O-glucuronide; F4: quercetin 3-O-glycoside; F5: kampferol 3-O-glycoside; F6: isorahamnetin 3-O-glucoside)

Packaging materials	Storage time	F1	F2	F3	F4	F5	F6	Total
		µg rutin/L						
Control ¹	00	0.50 ^a	0.76 ^{a,b,c}	2.69 ^a	3.05 ^{a,b,c}	0.59 ^{a,b,c}	0.49 ^{a,b}	8.09 ^{a,b}
PLA	0	0.43 ^{a,b}	0.82 ^{a,b}	2.44 ^{a,b}	3.24 ^{a,b}	0.68 ^{a,b}	0.50 ^{a,b}	8.11 ^{a,b}
	2	0.37 ^b	0.76 ^{a,b,c}	2.12 ^b	1.72 ^{c,d}	0.40 ^c	0.40 ^b	5.77 ^b
	4	0.41 ^{a,b}	0.58 ^c	2.10 ^b	1.74 ^{c,d}	0.42 ^c	0.40 ^b	5.64 ^b
	6	0.18 ^c	0.19 ^d	1.48 ^c	0.67 ^d	0.12 ^d	0.16 ^d	2.80 ^c
PET	0	0.47 ^{a,b}	0.94 ^a	2.79 ^a	3.77 ^a	0.80 ^a	0.57 ^a	9.34 ^a
	2	0.42 ^{a,b}	0.70 ^{b,c}	2.54 ^{a,b}	2.89 ^{a,b,c}	0.60 ^{a,b,c}	0.50 ^{a,b}	7.65 ^{a,b}
	4	0.43 ^{a,b}	0.70 ^{b,c}	2.32 ^{a,b}	2.16 ^{b,c}	0.47 ^{b,c}	0.47 ^{a,b}	6.54 ^b
	6	0.51 ^a	0.55 ^c	2.62 ^a	2.19 ^{b,c}	0.47 ^{b,c}	0.49 ^{a,b}	6.83 ^{a,b}

¹ sample unprocessed

Different letter in the same column indicate significant differences ($p < 0.05$).

Table 4: Hydroxycinnamoyl-tartrates and flavan-3-ols content in grape juice samples (mean values) during storage in PLA and PET bottles. (HTC1: 2-S-glutathionyl caftaric acid; HCT2: t-caftaric acid; HCT3: t-cautaric acid; HCT4: t-fertaric acid. Fla1: procyanidin B1; Fla2: catechin; Fla3: epigallocatechin gallate)

Packaging materials	Storage time	HCT1	HCT2	HCT3	HCT4	Total HCT	Fla1	Fla2	Fla3	Total Fla
		µg chlorogenic acid/L				µg catechin/L				
Control ¹	00	8.47 ^a	3.62 ^b	1.20 ^{a,b}	1.08 ^a	14.38 ^{a,b,c,d}	0.79 ^a	1.06 ^{a,b,c}	1.12 ^{b,c}	2.97 ^a
PLA	0	7.58 ^a	5.70 ^a	1.26 ^{a,b}	1.22 ^a	15.76 ^a	0.91 ^a	1.25 ^a	0.66 ^c	2.82 ^a
	2	7.49 ^a	5.59 ^a	1.23 ^{a,b}	1.22 ^a	15.53 ^a	0.81 ^a	0.75 ^{b,c}	1.77 ^{a,b}	3.33 ^a
	4	7.58 ^a	5.65 ^a	1.30 ^a	0.92 ^a	15.44 ^{a,b}	0.78 ^a	0.98 ^{a,b,c}	1.82 ^{a,b}	3.58 ^a
	6	7.55 ^a	5.57 ^a	1.13 ^{a,b,c}	1.07 ^a	15.32 ^{a,b}	0.36 ^b	0.59 ^c	2.54 ^a	3.49 ^a
PET	0	7.56 ^a	3.10 ^b	1.10 ^{b,c}	1.12 ^a	12.87 ^{b,c,d}	0.97 ^a	1.31 ^a	0.63 ^c	2.91 ^a
	2	7.11 ^a	2.94 ^b	1.03 ^c	1.06 ^a	12.14 ^{c,d}	0.90 ^a	1.16 ^{a,b}	1.18 ^{a,b}	3.23 ^a
	4	7.18 ^a	2.88 ^b	1.02 ^c	0.54 ^a	11.62 ^d	0.86 ^a	0.99 ^{a,b,c}	1.72 ^{a,b}	3.57 ^a
	6	7.81 ^a	3.55 ^b	1.20 ^{a,b}	1.18 ^a	13.74 ^{a,b,c,d}	0.85 ^a	0.85 ^{a,b,c}	1.84 ^{a,b}	3.54 ^a

¹ sample unprocessed

Different letter in the same column indicate significant differences ($p < 0.05$).

4. Conclusions

To the best of our knowledge, no shelf-life studies were ever been carried out on the storage in PLA bottles of acid and colored beverages like grape juice. As a whole, these results support the potential health properties of red grape juices owing to an elevated concentration of phenolic compounds, mainly anthocyanins, and antioxidant activity. The improved phenol content and antioxidant activity in juices after HHP define the potential application of this treatment in high-added-value fruit products. As expected, PLA packaging showed to be more sensible to the storage and a significant loss both in the phenol content and in the antioxidant activity was generally detected at 4 months of shelf-life, while samples in PET bottles were not significantly affected until the sixth months. Despite the many limitations and the need for further studies focused on improving PLA oxygen barrier properties by blending with bio-based oxygen scavengers or developing coating with high barrier materials, this study highlights the potential application of PLA as packaging for fruit juice with a short shelf-life.

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