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Changes in Physicochemical Properties of Wild and Cultivated Blackberry during Postharvest Cold Storage

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HIGHLIGHTS

- During 12 days of cold storage, the pH values of wild and cultivated blackberry samples were decreased.
- The titratable acidity was increased from 1.61 to 3.28 for wild blackberry, and from 2.07 to 3.25 for cultivated blackberry.
- The most suitable storage time of blackberry components was 12 days during cold storage at 5 °C.
- The wild blackberry was more resistant than cultivated one to cold storage.

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ABSTRACT

Background: Blackberry is a seasonal harvested fruit that is also very fragile and perishes quickly. A comparative study was conducted to find out influence of cold storage on physicochemical parameters of wild and cultivated blackberry over a period of 12 days.
Methods: The plant materials were composed of the blackberry fruit, including a compound of cultivated blackberry (*Rubus* spp.), and wild blackberry (*Rubus fruticosus* L. *agg*) which were harvested in the North of Morocco. The temperature of the storage of wild and cultivated blackberry was 5 °C for 4, 8, and 12 days, and then transferred to 25 °C for 1 day to simulate transport and commercialization. After that, the physicochemical parameters were analyzed. Statistical analyses were performed using SAS.
Results: During 12 days of storage, the pH of samples was decreased from 3.69 to 3.22 for wild blackberry, and from 4.85 to 3.43 for cultivated blackberry. The titratable acidity was increased from 1.61 to 3.28 for wild blackberry, and from 2.07 to 3.25 for cultivated blackberry. Flavonoids also showed a remarkable increase in values from 30 to 70.66 mg QE/100g of wild blackberries and from 25.33 to 60.66 mg QE/100g in cultivated ones between harvest and the last day of storage. The variation in skin color revealed a decrease during storage of L* brightness, a* redness, and yellowness b* for both blackberries.
Conclusion: The most suitable storage time of blackberry components during cold storage at 5 °C was 12 days. The temperature at 5 °C preserves the quality of the blackberry for both wild and cultivated ones. However, the wild blackberry was more resistant than cultivated one to cold storage.

Introduction

Over the past several years, researchers have interested in the conservation of fruits, especially seasonal and the fruits of small berries, which are fragile and perish quickly by their flesh marrow and also deprived core.

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Blackberry is a black or blue colored fruit produced by the mulberry tree, a thorny bramble of the genus *Rubus*. The wild blackberry (*Rubus fruticosus* L. agg.) is distributed worldwide between the 30° and 65° parallel of the Northern Hemisphere and between the 28° and 40° parallel of the Southern Hemisphere. It is very rich in antioxidants which play a considerable role in the prevention of certain cancers, and also has an anti-diabetic effect (Kaume et al., 2012). The blackberry is very rich in bioactive phenolic compounds, namely flavonoids, acidic phenolics, and tannins (Seeram, 2008), which in combination may help protect against obesity, inflammation, cardiovascular disease, cancer, diabetes, and other chronic diseases (Kraft et al., 2008; Liu et al., 2000; Prior et al., 2008; Shukitt-Hale et al., 2008).

Blackberries are generally harvested from July to September. It is a very sensitive fruit, which quickly perishes causing nutritional deterioration that can be harmful to our health. According to several recent studies, the best preservation of fresh blackberries is cold storage near harvest (Antunes et al., 2003; Perkins-Veazie and Kalt, 2002; Wu et al., 2010). Preservation by some methods such as chemical treatments, modified atmosphere, irradiation, etc. may lead to unfavorable changes. Cold storage preserves the phenolic compounds and nutritional values of fresh blackberries better than other techniques, with less deterioration of nutritional quality fruit depending on storage conditions and fruit maturity (Hassimotto et al., 2008).

In order to extend the shelf life after harvest and to protect the nutritional quality of the fruit, we investigated certain parameters which influence the structure and composition of the fruit. In this context, a comparative study was conducted to find out the influence of cold storage on physicochemical parameters of wild and cultivated blackberry over a period of 12 days.

Materials and methods

Plant materials

The plant materials were composed of the blackberry fruit, including a compound of cultivated (commercial) blackberry (*Rubus* spp.), and wild blackberry (*R. fruticosus* L. *agg*), which was harvested in the North of Morocco (35°47'16.3"N 5°54'31.3"W). Blackberries usually ripe without defects with a diameter between 1.2 cm and 1.5 cm were selected for wild blackberries and a diameter between 2.3 cm and 2.8 cm were selected for cultivated blackberries.

Design of experimental groups

Both wild and cultivated blackberries were packaged in 250 ml plastic jars (15 fruits per jar), and stored at 5 °C with 90-95% Relative Humidity (RH) for 12 days. To calculate the weight loss, each sachet was weighed before storage. After 4, 8, and 12 days of storage at 5 °C, the blackberries were transferred to 25 °C and 70% RH and changes in the properties of the fruit were evaluated after 1 day of storage at 25 °C. Only 1-day stored sample (at 25 °C) was tested because the fruit is very perishable. We have considered the start day (0) of the samples as a control. After harvest, the blackberry samples were stored at 5 °C for 4, 8, and 12 days; and at 25 °C for 4+1, 8+1, 12+1 days. On the other word, we considered days 0, 4, 8, and 12 as cold storage days, while 4+1, 8+1, and 12+1 days were considered as shelf life conditions.

Determination of analytical parameters

-Color

The skin color was measured with a colorimeter (Chroma Meter CR-400, Konica Minolta, China) that analyzed the spectral distribution of the color (the parameters L*, a*, and b*).

-pH

The potentiometric method of the AOAC (1990) was used. For this purpose, 50 ml of distilled water were added to 5 g of blackberries; after 10 min homogenization, pH of the solution was determined.

-Titratable acidity

The titratable acidity (in mEq/100g) was specified with the colorimetric method.

-Soluble dry extract

The measurement was performed using a digital hand-held refractometer (HI96801, China). Drops of blackberry juice were used to measure the Brix degree after 5 s.

-Total sugar content

The total sugars (mg/g) were measured based on the method of Dubois et al. (1956).

-Total polyphenols

The determination of total polyphenols (mg Eq A.G/g fresh matter) was carried out by adding 50 ml of distilled water to 5 g of blackberries (Wood et al., 2002).

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-Total flavonoids

The total flavonoids (mg QE) were determined according to Marinova et al. (2005) with some modifications using a visible UV spectrophotometer (Evolution 201, Germany) at 510 nm.

-Anthocyanins

The dosage of anthocyanins from extracts of blackberry was determined by the differential pH method (Al-Farsi et al., 2005; Wrolstad et al., 2005). The pH-differential method is based on the change of the structure of the anthocyanin chromophore between pH 1.0 and 4.5. The monomeric anthocyanins undergo a reversible structural transformation as a function of pH (colored form of oxonium at pH 1.0 and colorless hemifacial form at pH 4.5). The difference in the absorbance of these pigments at 520 nm (max visible λ of anthocyanins) is proportional to the concentration of the dye. The content of total anthocyanins was measured using two buffers of potassium chloride and sodium acetate; the dilution factor was determined by diluting the test portion with a pH 1.0 buffer, the absorbance must be seen between 0.2 and 1.4 IU; the absorbance of the sample diluted with the buffer pH 1.0 and pH 4.5 was determined both at 520 and 700 nm. The absorbance of the diluted sample (A) was calculated as follows:

A=(A520 nm-A700 nm) pH 1.0-(A520 nm-A700 nm) pH 4.5

Concentration of pigments in monomeric anthocyanins in the initial sample was calculated using the following formula:

Monomeric anthocyanin pigment (mg/l)=(A×MW×DF×1000)/(ε×)

Where A: absorbance of the sample; MW: molar mass of cyanidine 3-glucoside=449.2 g/mol; DF: sample dilution factor; ε: molar extinction coefficient=26 900 L/mol; 1 000: the conversion factor from g to mg; l:=1cm.

-Vitamin C

The determination of vitamin C is carried out by 2,6 dichlorophenol-indophenol (DCPIP). Vitamin C is titrated with a 2,6 dichlorophenol-indophenol solution (9.32×10^{-4} mol/L), until a persistent pink color appears for 30 s. The vitamin C content was determined by the following formula**:**

[Vitamin C] (g/l)=[DCPIP]×V×M/V0

Where DCPIP: concentration of DCPIP in mol/l; V: DCPIP volume in ml; M: Molar mass of vitamin C=176.1242 g/mol; V0: Sample volume in ml.

-Texture changes over time

The samples were measured with a texture analyzer (Ametek, TA1, Germany). A perforation test and a texture profile analyses were carried out with 150 g of fruit for each test. The perforation was carried out on the surface of the fruit. Three indices were considered: 1) the fruit breaking strength (elastic limit) as the maximum strength (N) reached during the test; 2) the hardness of the flesh, such as the area (N/s) calculated between the starting point and the maximum force; 3) the stiffness gradient (N/s) calculated between the starting point and the break-even point. Texture profile analyses were performed on the face of whole fruits in compression mode. Also, the hardness, cohesion, softness, and elasticity were evaluated.

Statistical analysis

All analysis were done in triplicate and represented as means±standard deviation were calculated of triplicate measurements. Statistical analyses were performed using the statistical software SAS (Version 9.1. 2002).

Results

Several quality parameters of blackberries were examined at the initial sampling and during storage periods (Tables 1-4). During 12 days of storage, the pH of samples was decreased from 3.69 to 3.22 for wild blackberry, and from 4.85 to 3.43 for cultivated blackberry. The titratable acidity was increased from 1.61 to 3.28 for wild blackberry, and from 2.07 to 3.25 for cultivated blackberry. Flavonoids also showed a remarkable increase in values from 30 to 70.66 mg QE/100g of wild blackberries and from 25.33 to 60.66 mg QE/100g in cultivated ones between harvest and the last day of storage. The variation in skin color revealed a decrease during storage of L* brightness, a* the redness, and the yellowness b* for both blackberries.

Discussion

In this study, we determined quality parameters of wild and cultivated blackberry stored at cold temperature. Our results are similar to the study by Fadda et al. (2015) who examined the antioxidant activity of strawberry tree fruits during cold storage. Perkins-Veazie and Kalt (2002) showed that blackberries presented the same analytical parameters after storage for 3 and 14 days at 2 °C under pressure (15 kPa CO_2, 10 kPa O_2). Segantini et al. (2017) found that blackberries, cultivated with their

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Table 1: The effect of storage time on pH, titratable acidity, total soluble solids, Vitamin C, and weight loss of wild and cultivated blackberry

Storage time (days)	pH		Titratable acidity (mEq/g)		Total soluble solids (Brix)		Vitamin C (mg/100g)		Weight loss (%)	
	W.B	C.B	W.B	C.B	W.B	C.B	W.B	C.B	W.B	C.B
Control	3.69 ^{e}	4.85 ^{a}	1.61	2.07 ^{f}	13.68 ^{d}	8.00 ^{e}	20.13 ^{a}	15.40 ^{c}	0.00 ^{l}	0.00 ^{l}
4	3.24 ^{g}	4.22 ^{c}	2.33	2.87 ^{d}	15.53 ^{c}	8.33 ^{e}	18.56 ^{b}	15.03 ^{c}	0.12 ^{k}	0.98 ^{f}
4+1	3.20 ^{g}	3.85 ^{d}	2.91	2.97 ^{cdb}	13.50 ^{d}	8.66 ^{e}	13.53 ^{d}	13.13 ^{e}	0.14 ^{k}	1.76 ^{e}
8	3.55 ^{ef}	4.54 ^{b}	3.14	3.11 ^{abcd}	17.50 ^{b}	8.93 ^{e}	13.96 ^{cd}	11.30 ^{f}	0.22 ^{j}	1.83 ^{d}
8+1	3.46 ^{f}	4.21 ^{c}	3.18 ^{abc}	3.02 ^{abcd}	13.20 ^{d}	9.00 ^{e}	12.26 ^{e}	8.80 ^{g}	0.31 ^{i}	2.34 ^{c}
12	3.13 ^{g}	3.56 ^{e}	3.22 ^{a}	3.15 ^{abcd}	19.16 ^{a}	9.33 ^{e}	13.26 ^{d}	10.77 ^{f}	0.40 ^{h}	3.21 ^{b}
12+1	3.22 ^{g}	3.43 ^{f}	3.28 ^{a}	3.25 ^{a}	13.00 ^{d}	9.66 ^{e}	11.33 ^{f}	8.67 ^{g}	0.49 ^{g}	3.66 ^{a}
Significance	***	***	***	***	***	***	***	***	***	***

-0, 4, 8, and 12 are cold storage days, while 4+1, 8+1, and 12+1 are days of conditioned storage; Wild Blackberry (W.B) and Cultivated Blackberry (C.B); data followed by different letters within each column are means with the same letter are not significantly different within each column are significantly different by Tukeys test; significance *p<0.05 ; ** p<0.01, *** p<0.001; NS: Not Significant

Table 2: The effect of storage time on total polyphenols, flavonoids, total anthocyanins, and sugars of wild and cultivated blackberry

Storage time (days)	Total polyphenols (mg/100g)		Flavonoids (mg QE/100g)		Total anthocyanins (mg/100g)		Sugars (mg/100g)	
	W.B	C.B	W.B	C.B	W.B	C.B	W.B	C.B
0	135.33 ^{f}	83.00 ^{j}	30.00 ^{h}	25.33 ^{i}	133.67 ^{f}	114.70 ^{h}	3.88 ^{h}	3.95 ^{g}
4	149.66 ^{e}	93.33 ^{i}	34.66 ^{g}	36.66 ^{g}	136.00 ^{f}	125.30 ^{g}	4.12 ^{e}	4.12 ^{e}
4+1	150.0 ^{e}	103.30 ^{h}	40.66 ^{f}	40.00 ^{f}	143.00 ^{e}	132.67 ^{f}	3.73 ^{i}	3.74 ^{i}
8	189.33 ^{c}	105.00 ^{h}	45.33 ^{e}	44.00 ^{e}	160.67 ^{c}	152.00 ^{d}	4.23 ^{d}	4.21 ^{d}
8+1	194.67 ^{c}	116.00 ^{g}	58.00 ^{bc}	54.66 ^{d}	165.33 ^{b}	161.67 ^{bc}	4.04 ^{f}	3.17 ^{k}
12	209.33 ^{b}	133.30 ^{f}	69.00 ^{a}	56.00 ^{dc}	170.33 ^{a}	165.67 ^{b}	4.80 ^{a}	4.31 ^{c}
12+1	220.33 ^{a}	157.30 ^{d}	70.66 ^{a}	60.66 ^{b}	174.00 ^{a}	170.33 ^{a}	4.43 ^{b}	3.52 ^{j}
Significance	***	***	***	***	***	***	***	***

-0, 4, 8, and 12 are cold storage days, while 4+1, 8+1, and 12+1 are days of conditioned storage; Wild Blackberry (W.B) and Cultivated Blackberry (C.B); data followed by different letters within each column are means with the same letter are not significantly different within each column are significantly different by Tukeys test; significance *p<0.05 ; ** p<0.01, *** p<0.001; NS: Not Significant

Table 3: The effect of storage time on the color parameters of blackberry fruits

Storage time (days)	Wild blackberry					Cultivated blackberry				
	L*	a*	b*	(a^2+b^2)	$(\tan^{-1}b^*/a^*)$	L*	a*	b*	(a^2+b^2)	$(\tan^{-1}b^*/a^*)$
0	49.74 ^{a}	6.61 ^{b}	1.84 ^{ab}	6.86	15.55	47.68 ^{a}	25.73 ^{a}	7.32 ^{a}	26.75	15.88
4	48.95 ^{a}	5.92 ^{b}	1.59 ^{ab}	6.12	15.03	38.01 ^{cab}	15.97 ^{ab}	4.12 ^{ab}	16.49	14.46
4+1	43.3 ^{ab}	4.99 ^{b}	0.89 ^{b}	5.06	10.11	31.49 ^{cab}	14.22 ^{ab}	2.87 ^{ab}	14.50	11.41
8	35.87 ^{cab}	4.74 ^{b}	0.37 ^{b}	4.75	4.46	27.07 ^{cab}	13.63 ^{ab}	3.16 ^{ab}	13.99	13.05
8+1	34.30 ^{bcd}	2.51 ^{b}	0.99 ^{ab}	2.69	21.52	17.62 ^{cd}	11.47 ^{ab}	2.45 ^{ab}	11.72	12.05
12	24.91 ^{cdb}	2.11 ^{b}	1.12 ^{ab}	2.38	27.95	15.02 ^{d}	8.88 ^{b}	1.91 ^{ab}	9.08	12.13
12+1	19.67 ^{cd}	1.19 ^{b}	1.23 ^{ab}	1.71	54.27	14.38 ^{d}	6.41 ^{b}	3.14 ^{ab}	7.13	26.11
Significance	**	*	NS			**	*	NS		

-0, 4, 8, and 12 are cold storage days, while 4+1, 8+1, and 12+1 are days of conditioned storage; data followed by different letters within each column are means with the same letter are not significantly different within each column are significantly different by Tukeys test; significance *p<0.05 ; ** p<0.01, *** p<0.001; NS: Not Significant

Table 4: The effect of storage time on the texture parameters of blackberry fruits

Storage time (days)	Texture test (N)	
	W.B	C.B
Control	331.67 ^{a}	302.69 ^{a}
4	310.60 ^{a}	299.30 ^{b}
4+1	309.55 ^{ab}	288.36 ^{c}
8	307.35 ^{cb}	220.59 ^{d}
8+1	306.27 ^{cd}	217.20 ^{e}
12	304.33 ^{d}	212.00 ^{f}
12+1	304.00 ^{d}	210.82 ^{f}
Significance	***	***

-0, 4, 8, and 12 are cold storage days, while 4+1, 8+1, and 12+1 are days of conditioned storage; Wild Blackberry (W.B) and Cultivated Blackberry (C.B); data followed by different letters within each column are means with the same letter are not significantly different within each column are significantly different by Tukeys test; significance *p<0.05 ; ** p<0.01, *** p<0.001; NS: Not Significant different genotypes, showed some differences in pH and titratable acidity which is consistent with our findings. However, opposite finding was reported in the research by Wu et al. (2010) about effects of refrigerated storage on antioxidant capacities of 'Marion' and 'Evergreen' blackberries. Furthermore, Perkins-Veazie et al. (1996) found that cultivated blackberries have less decrease in titratable acidity than other cultivated blackberries as well as the results of the study by Kim et al. (2015). Our finding could be explained by the presence of the plolyphenoloxidase enzymatic process in that both blackberry species is linked with the phenomenon of co pigmentation. Also, the increase in titratable acidity did not affect the general flavor peaks, and cold storage had advantages on quality of our blackberry samples as reported by Perkins-Veazie and Kalt (2002).

The soluble solids content of two blackberry fruits did not change during conditioned storage, which largely explains the balanced ratio between fruit maturity and sugar. However, on cold storage, we observed a slight increase which was due to the ratio of weight loss that concentrates cellular sap which is in agreement with findings by Salgado and Clark (2016), Wu et al. (2010), and Segantini et al. (2017). However, contrary to our results, Perkins-Veazie et al. (1996) and Kim et al. (2015) affirmed the decrease in soluble solids in cultivated blackberries. In the current investigation, vitamin C values were decreased during storage of both fruits with significantly different data between wild and cultivated blackberries, and the wild fruit had more vitamin C than the cultivated fruits. The decrease in vitamin C can be explained by its degradation during conditioned storage (4+1, 8+1, 12+1 days) at 25 °C and in cold storage (4, 8, 12 days) at 5 °C which is in accordance with reports by Yilmaz et al. (2009). However, our results are different from other studies on blackberries which showed ascorbic acid was increased during storage (Fadda et al., 2015; Orak et al., 2012).

We found that weight loss of the blackberries was overcome after 12 days of storage which is in agreement with Guerreiro et al. (2013) and Fadda et al. (2015). Perkins-Veazie et al. (1996) revealed that the weight loss of different types of cultivated blackberries varies from 0.8 to 3.3% after storage. While Kim et al. (2015) found 2-4% in the whole transformation of cultivated blackberries which are stored at 20 °C due to higher respiration compared to 1 °C. Segantini et al. (2017) explained that weight loss in cultivated blackberries concentrates cellular sap, which leads to a slight increase in soluble solid. The weight loss of wild and cultivated blackberries during conditioned storage was significantly greater than that observed during cold storage. This finding explains why cold storage preserves the fruit better than conditioned storage.

We harvested fruit at the stage of full maturity; therefore, we suggest that the over-ripening process is responsible for the increase in total polyphenols of blackberries during cold and conditioned storage which is similar to the results by Kim et al. (2015). In the present work, flavonoids also showed a remarkable increase in values from 30 to 70.66 mg QE/100g of wild blackberries and from 25.33 to 60.66 mg QE/100g in cultivated ones between harvest and the last day of storage. The total polyphenols and flavonoids of the wild blackberry are more remarkable than the cultivated blackberry. This increase in these compounds after storage can be due to stress conditions such as low temperature during cold storage and conditioned storage after harvest. Out findings for flavonoids are similar to those by Segantini et al. (2017) who noted that the level of flavonoids increase after storage to 53%.

In storage of red oranges at 4 °C, the level of anthocyanins increases eight times compared to fruits stored at 25 °C as shown previously by Lo Piero et al. (2005). Šamec and Piljac-Žegarac (2011) reported that blackberries of the species (*R. fruticosus*) gained 15 to 20% of the total anthocyanin when stored for 4 days at 4 or 25 °C. The increase in anthocyanin in cultivated blackberries may be due to the firmness of these fresh market fruits compared to the processed fruit reported by Wu et al. (2010). The increase of the antioxidant parameters at the end of the storage period of the two samples shows that the cold room has beneficial and similar effect with the same fruit with different species (De Souza et al., 2018).

The variation in skin color revealed a decrease during storage of L* brightness, a* the redness, and the yellowness b* for both wild blackberries and cultivated samples (Table 3). The L* and a* brightness parameters decreased significantly after 4 days and one day of conditioned storage for wild blackberries and for cultivated blackberries just after 4 days of cold storage. It was observed that wild blackberries retain their skin color more than the cultivated ones. The decrease in skin color during cold storage may be due to enzymatic browning for both samples (Guerreiro et al., 2013). The L*, a*, and b* data were all considerably reduced during storage, which is in agreement with the results found by Fadda et al. (2015).

Our texture results showed a significant lowering of the firmness of both fruit types, especially the cultivated one (Table 4). A decrease in the firmness of the cultivated fruit rather than the wild fruit may be due to the environmental conditions and the nutritional state of the plantations. These results are identical to that of another study carried out by Yilmaz et al. (2009) on the physicochemical characteristics of wild and cultivated blackberry fruits from Turkey. These results are also similar to the texture

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of the strawberry fruit after storage according to previous studies (Cordenunsi et al., 2003; Guerreiro et al., 2013). Segantini et al. (2017) noted that different blackberries had a firmness range of 4.9-9.0 N at harvest; but after storage, the firmness was 4.0 to 10.1 N, except that the genotype of A2491 that had a firmness reduced like our results. This suggests that the weight loss was negatively correlated with firmness so that the firmer the blackberry, the more potential for postharvest storage. The cultivated blackberry exhibits more weight loss than the wild one, in addition its firmness was also weak compared to the wild one. It can be concluded from this data that the wild blackberry is resistant to conditioned storage better than the cultivated blackberry.

Conclusion

The most suitable storage time of the blackberry components was 12 days during cold storage at 5 °C. The temperature at 5 °C preserves the quality of the blackberry for both wild and cultivated ones. However, the wild blackberry was more resistant than cultivated one to cold storage. It should be more beneficial to store blackberries at temperatures around 5 °C during marketing, in order to maintain suitable shelf life. In future, further analysis of the sensory properties of the blackberry will provide additional information about their suitable storage time.

Author contributions

N.Ag., N.Az., S.Z., and A.M. designed the study; N.Ag., S.Z., and N.Az. conducted the experimental work; N.Az. analyzed the data; N.Az., A.M., and M.R.B wrote the manuscript. All the authors read and approved the final manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

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