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Ready-to-eat raspberries: qualitative and nutraceutical characteristics during shelf-life

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Abstract: Raspberry (*Rubus idaeus* L.) fruits are characterised by a high content of nutraceuticals, such as vitamin C, polyphenols and anthocyanins, which are considered antioxidant compounds. The ready-to-eat raspberry product could increase the market opportunities and the consumption of this high-value-added fruit. The aim of this research was to evaluate the evolution of qualitative and nutraceutical characteristics during the shelf-life of ready-to-eat raspberries. Samples from three raspberry cultivars ('Glen Magna', 'Tulameen' and 'Heritage') were sanitized and then packed in polypropylene bowls. The analyses were carried out at harvest (raw material) and after 3, 6 and 8 days of storage at 3°C. The study indicated the loss of fruit firmness as the most problematic aspect, followed by a less important change in hue values from light red to dark red. The modifications of chemical-physical parameters (soluble solids content, pH and titratable acidity) during shelf-life did not compromise the product quality. Processing and cold storage affected only slightly the nutraceutical profile (scavenging activity, phenols and anthocyanin content), except for ascorbic acid, therefore, the ready-to-eat raspberries could be considered a good source of compounds with potential health benefits. Some handling difficulties were highlighted during processing due to the high fragility of fruit which caused a high percentage of waste.

1. Introduction

Increasing epidemiological data suggested the correlation between the consumption of fresh fruits and vegetables with the prevention, delay or onset of chronic degenerative diseases, including cancer (Kaur and Kapoor, 2001). Fruit and vegetables are, in fact, rich of the so-called

nutraceuticals, which are compounds with a significant biological action, especially as antioxidants (Szeto *et al.*, 2002; Fu *et al*., 2011). Phenolics compounds, as an example, have a significant antioxidant activity (Szajdek and Borowska, 2008). They can donate electrons to the reactive oxygen species (ROS), converting them into innocuous molecules (Haminiuk *et al.*, 2012) and they can also exert beneficial modulatory action in cells (Williams *et al.*, 2004). Ascorbic acid (AA) is an essential vitamin that is found in fruits and vegetables and it has several positive functions: in plant and animal systems, AA interacts enzymatically and non-enzymatically with the reactive oxygen species and it is able to terminate the radical chain reactions by converting the ROS in non-toxic products (Davey *et al.*, 2000).

Red raspberry fruit (*Rubus idaeus* L.) are highly appreciated by consumers for their aromatic taste, especially when they are fully ripe. They are an important dietary source of bioactive compounds (Kähkönen *et al.*, 1999) such as anthocyanins, hydrosoluble pigments belonging to the class of polyphenols (Benvenuti *et al.*, 2004; Pantelidis *et al.*, 2007; Szajdek and Borowska, 2008) and vitamin C (Mazur *et al*., 2014 a, b). Unfortunately the postharvest life of this small fruit is short, because of its high respiration rate, loss of firmness and freshness, susceptibility to fruit rot and darkening (Krüger *et al.*, 2011); consequently it loses very early its market viability.

Ready-to-eat fruits and vegetables are one of the most important novel products introduced into the Italian agro-food system over the last 30 years and they represent a dynamic and innovative sector. In the last few years, the consumption of these products has increased, because the doubt and mistrust that characterised them in the 90's have now disappeared. Ready-to-eat products are very convenient because they do not need any preparatory operation (washing, cutting) before consumption. There are obviously some disadvantages in using this kind of products: they are usually costly and the processing operation can shorten the postharvest life of the fruit/vegetable. However, since fruit are poorly eaten by kids and teens (OECD, 2012) the diffusion of ready-to eat products is often encouraged in order to increase fruit consumption among young people. Raspberries are generally eaten fresh and a ready-to-eat product could increase the consumption of this bioactively rich fruit. The aim of this research was to evaluate the qualitative and nutraceutical characteristics during the shelf-life of ready-to-eat raspberries.

2. Materials and Methods

Raw material

Investigations were carried out on raspberry fruits of three different cultivars picked at commercial maturity: 'Heritage' and 'Glen Magna', grown at the fruit experimental centre of Milan University (Italy) and 'Tulameen', grown at the experimental comparative field for berry fruits of the Catholic University of Piacenza (Italy). On arrival at the laboratory the fruits were stored at 3±1°C till the processing time, always within 24 h.

Processing

Raspberries were manually selected in order to remove damaged fruits. Fruits were dipped in a disinfectant solution (0.022% of active chlorine) for 10 min and successively rinsed in tap water for 1 minute. Raspberries, after being softly dried with adsorbent paper, were packed and hermetically sealed in polypropylene (Transpiration rates (TR): O_2TR: 0.117 $mm^3\ m^{-2}\ d^{-1}\ Pa^{-1}$; CO_2TR: 1.847 $mm^3\ m^{-2}\ d^{-1}\ Pa^{-1}$) bowls (100 g per bowl), previously sanitized by UV radiation, and hermetically sealed with a film (O_2TR: 0.476 $mm^3\ m^{-2}\ d^{-1}\ Pa^{-1}$; CO_2TR: 2.17 $mm^3\ m^{-2}\ d^{-1}\ Pa^{-1}$) using a packaging machine Mod. TSM 95 (MINIPACK-TORRE, Dalmine, Bergamo, Italy). A rack, made of the same material was inserted inside the bowl in order to drain any dripping liquid. The packed fruits were then stored at 3±1°C for 8 days.

Chemical and physical analyses

Chemical and physical analyses were carried out on six samples (100 g each) of raspberry fruits per treatment at harvest (raw material) and after 3, 6 and 8 days of storage at 3°C. Fruit of each bowl were weighed and the amount of weight loss was measured by the percentage alteration between the initial (packaging) and final weight of the fruit of each bowl. Tititratable acidity [g citric acid/100 g fresh weight (FW)] and pH were assessed according to the Official Methods of Analysis (AOAC, 1985) and the soluble solids content (SSC, %) was measured by a multiscale automatic refractometer (RFM91 model, BS, UK). Colour parameters L*, a* and b* were measured by using the spectrophotometer CM-2600-D Minolta and hue was calculated as arctangent (b*/a*). Texture was measured on 15 fruit per treatment and sampling date, using an Instron Universal Testing Machine (model 1140, Instron, High Wycombe, UK), as maximum force (g) used to compress by the fruit (crosshead speed 10 cm/min).

Analysis of the nutritional compounds

Nutritional compounds were analyzed on 3 samples (100 g each) per treatment and sampling date. The fruit extracts (2 per sample) were prepared as follows: 2 g of homogenized flesh were extracted with 18 mL of solution made of EtOH 95% and HCl 0.02N (1:1 v/v), vortexed for 30 sec, centrifuged (15 min, 4°C, 10000 g) and filtered through a cheesecloth.

Since the most important nutraceuticals in *Rubus* fruits belong to the phenols, this class of compounds was analysed by two different assays.

i) Total phenols content (TPC) was measured by Folin-Ciocalteu method, as described by Singleton and Rossi (1965). An aliquot of 150 µL of fruit extract, 5 mL of distilled water and 1 mL Folin-Ciocalteu reagent were incubated for 8 min, followed by the addition of 2 mL of 20% Na_2CO_3. After 2h in the dark, the absorbance was measured at 730 nm against blank with a UV-UVIDEC 320 spectrophotometer (Jasco, Japan). Results were expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

ii) Total monomeric anthocyanin pigment was estimated by the pH differential method described by Giusti and Wrolstad (2001) using a UV-UVIDEC 320 spectrophotometer (Jasco, Japan). Extracts were diluted 1:10 with two buffers at pH 1.0 and 4.5; readings of each sample were made at 510 nm and 700 nm and the absorbance (A) of the diluted sample was calculated using the formula:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$$

The anthocyanin concentration was calculated using the cyanidin 3-glucoside (C3G) molar extinction coefficient 26,900 and it was expressed as mg of C3G equivalents in 100 g of FW.

Ascorbic acid (AA) was determined according to the method described by Davey *et al.* (2003) with some modifications. Briefly, 300 mL of the extract were added to 600 mL of 6% meta-phosphoric acid solution, diluted 1:20 in ortophosphoric acid solution (0.02M) and analyzed by an HPLC system (Jasco, Japan). AA was separated on an Inertsil ODS-3 (GL Science) column kept at 37°C and connected to a variable wavelength detector (UV- 1575, Jasco) set at 254 nm, using as mobile phase 0.02M orthophosphoric acid at the flow rate of 0.7 mL/min. Ascorbic acid concentration was expressed as mg in 100 g of FW.

Samples were also subjected to one of the most used free radical scavenging assays, which is relatively easy and cheap in execution and is strongly correlated with the sample's nutraceutical content: the 2,2-diphenyl-1-pycrilidrazyl ($DPPH^{\cdot}$) assay (Brand-Williams *et al.*, 1995). The amount of 500 µL of a solution 0.5 mM DPPH (dissolved in EtOH) 2.0 mL EtOH and 100 µL of sample was mixed in a 1-cm path cuvette and the absorbance at 517 nm against blank (2.5 mL EtOH and 100 µL of sample) was recorded at time 0 s and 180 s. The percentage of DPPH decrease was computed as:

$$\text{DPPH } \% = [(A_{t0} - A_{t180})/A_{t0}] \times 100$$

and the results were expressed as mg Trolox equivalents in 100 g of FW.

Statistical analysis

Statistical analyses were carried out with the Statgraphics software v.5.1 package (Manugistics, Rockwell MD). Data were submitted to multifactor ANOVA evaluating the main effects of the factors "cultivar" and "shelf-life". Differences among the treatments were determined by Tukey multiple range test or by Least significant distance (LSD) test ($P \leq 0.05$). Data were also examined using multivariate analysis by Principal Component Analysis (PCA).

3. Results

At the end of the shelf-life of the ready-to-eat raspberries, a fairly limited weight loss (0.1-0.3%), was noticed in all samples, mostly due to a slight juice dripping (data not shown).

Samples from different cultivars were characterized by different soluble solids content (Table 1): the cultivar Tulameen showed higher SSC values, followed by 'Heritage' and 'Glen Magna'. During shelf-life, this parameter decreased slightly in the cultivars Tulameen and Glen Magna while it did not change in the 'Heritage' fruit.

Total acidity decreased during shelf-life in 'Heritage' and 'Glen Magna' and slightly in 'Tulameen' (Table 1). No important changes in pH values were shown by 'Heritage' and 'Tulameen', while a significant decrease of pH was found in 'Glen Magna' fruit during the storage at 3°C (Table 1).

The compression test showed similar firmness for all the cultivars (Table 1). After only 3 days of shelf-life at 3°C, firmness decreased markedly in 'Heritage' (-32%) but only slightly in 'Tulameen' and 'Glen Magna' (Table 1). At the day 6 a significant decrease in firmness was observed in all the cultivar and, at end of the shelf-life (8 days) all the fruits had a firmness loss between -43% and -48%.

Table 1 - Maturity indices of ready-to-eat fruit from different raspberry cultivars, at harvest (raw) and after 3-6-8 days shelf-life at 1°C

Cultivar	Day of shelf-life	pH	SSC (%)	Acidity (g citric acid/100 g FW)	Firmness (g)
Heritage	raw	2.66 a	10.78 a	2.13 b	14.38 b
	3	2.62 a	10.68 a	2.03 a	9.82 a
	6	2.61 a	10.47 a	2.01 a	8.75 a
	8	2.67 a	10.49 a	1.97 a	7.46 a
Tulameen	raw	2.77 a	11.52c	1.55 b	14.50 b
	3	2.71 a	11.13 b	1.55 b	12.29 b
	6	2.75 a	10.83 ab	1.52 b	9.30 a
	8	2.76 a	10.74 a	1.38 a	8.23 a
Glen Magna	raw	2.82 a	9.54 b	1.72 c	15.05 b
	3	2.82 a	9.31 ab	1.56 b	11.89 ab
	6	2.90 ab	9.18 ab	1.49 ab	10.13 a
	8	2.97 b	9.01 a	1.42 a	8.63 a
Main Factors					
Cultivar	Heritage	2.64 a	10.60 b	2.03 b	10.13 a
	Tulameen	2.75 b	11.05 c	1.50 a	11.07 a
	Glen Magna	2.87 c	9.26 a	1.54 a	11.42 a
Day of shelf-life	raw	2.74 a	10.61 b	1.80 c	14.64 c
	3	2.71 a	10.37 ab	1.71 b	10.97 b
	6	2.75 a	10.10 a	1.67 b	9.74 ab
	8	2.80 a	10.13 a	1.58 a	8.10 a

Different letters indicate significant differences among the days of shelf-life of the same cultivar or among the main factors (Tukey test).

Visual appearance is a key issue for commercial shelf-life as consumer acceptance is often based on fruit colour which is used to make conclusions on the freshness of the product. The colour of 'Heritage' and 'Tulameen' fruits was characterized, at harvest, by lower L*, a* and b* values (Table 2) than those reported in literature (Çekic and Özgen, 2010; Krüger *et al.*, 2011). Fruit of 'Glen Magna' showed higher lightness (L*) and lower a* and hue values than the other two cultivars (Table 2). During shelf-life, the trend of L*, a* and b* values was not well defined, while hue decreased significantly in 'Glen Magna' and 'Heritage' indicating a darker red colour of the fruit. 'Tulameen' sample was, instead, more stable from the chromatic point of view.

At harvest the amounts of total anthocyanin of 'Heritage' (Granelli *et al.*, 2010), 'Tulameen' (Kruger *et al*., 2011) and 'Glen Magna' (Mazur *et al.*, 2014 b) were within the range of the values reported in literature (Fig. 1A) and the cv. Heritage showed the highest content. The cold storage induced a slight but significant loss of anthocyanin in all samples: after only 3 days (-20%) for 'Tulameen' and after 8 days for 'Heritage' (-12%) and 'Glen Magna' (-13%).

The average total polyphenols content (Fig. 1B) was higher in 'Glen Magna', followed by 'Tulameen' and 'Heritage'. TPC resulted quite stable over the shelf-life and no significant decrease was recorded. In our study the 'Tulameen' fruits showed the highest ascorbic acid content (Fig. 1C) at the harvest. AA content decreased significantly after only 3 days in in 'Tulameen' fruit which showed a loss of 26%. The final losses were about 26% for 'Heritage' and 'Tulameen' and even higher (-42%) for 'Glen Magna'.

The cultivar Heritage showed a significantly lower scavenging activity value (Fig. 1D) than the other cultivar at harvest and this difference was confirmed over the shelf-life. During storage the free radical scavenging activity had an up and down trend, and the end of the shelf-life it resulted slightly, even though significantly, decreased only in the 'Tulameen' samples.

Principal components analysis (PCA) can provide an overview of the shelf-life behaviour of ready-to-eat raspberries from different cultivars (Fig. 2). From the PCA analysis of the raspberry samples three functions were extracted, explaining the 91.2% of total variance. Considering the first two principal components PC1 (54.3% of the total variance) grouped the

Table 2 - Color parameters of ready-to-eat fruit from different raspberry cultivars, at harvest (raw) and after 3-6-8 days shelf-life at 1°C

	Day of shelf-life	a*	b*	L*	Hue (h°)
Heritage	raw	21.75 ab	9.75 a	27.44 ab	24.03 b
	3	22.84 ab	9.10 a	27.60 ab	21.58 a
	6	21.65 a	8.23 a	26.20 a	20.73 a
	8	24.38 b	9.67 a	29.77 b	21.54 a
Tulameen	raw	18.90 a	8.36 a	29.51 a	23.64 a
	3	20.53 a	8.99 a	29.83 a	23.58 a
	6	19.23 a	8.20 a	29.33 a	23.07 a
	8	20.48 a	8.90 a	30.31 a	23.44 a
Glen Magna	raw	18.17 a	7.25 a	30.83 b	21.89 b
	3	19.76 a	7.12 a	28.18 a	19.45 a
	6	19.13 a	6.83 a	28.96 a	19.53 a
	8	19.34 a	6.43 a	31.30 b	18.32 a
Main Factors					
Cultivar	Heritage	22.65 b	9.18 b	27.75 a	21.96 b
	Tulameen	19.78 a	8.61 b	29.74 b	23.43 c
	Glen Magna	19.09 a	6.90 a	29.81 b	19.79 a
Day of shelf-life	raw	19.60 a	8,45 a	29,25 ab	23,18 b
	3	21.04 ab	8.39 a	28.53 a	21.53 a
	6	20.00 ab	7.75 a	28.16 a	21.11 a
	8	21.40 b	8.33 a	30.45 b	21.09 a

Different letters indicate significant differences among the days of shelf-life of the same cultivar or among the main factors (Tukey test).

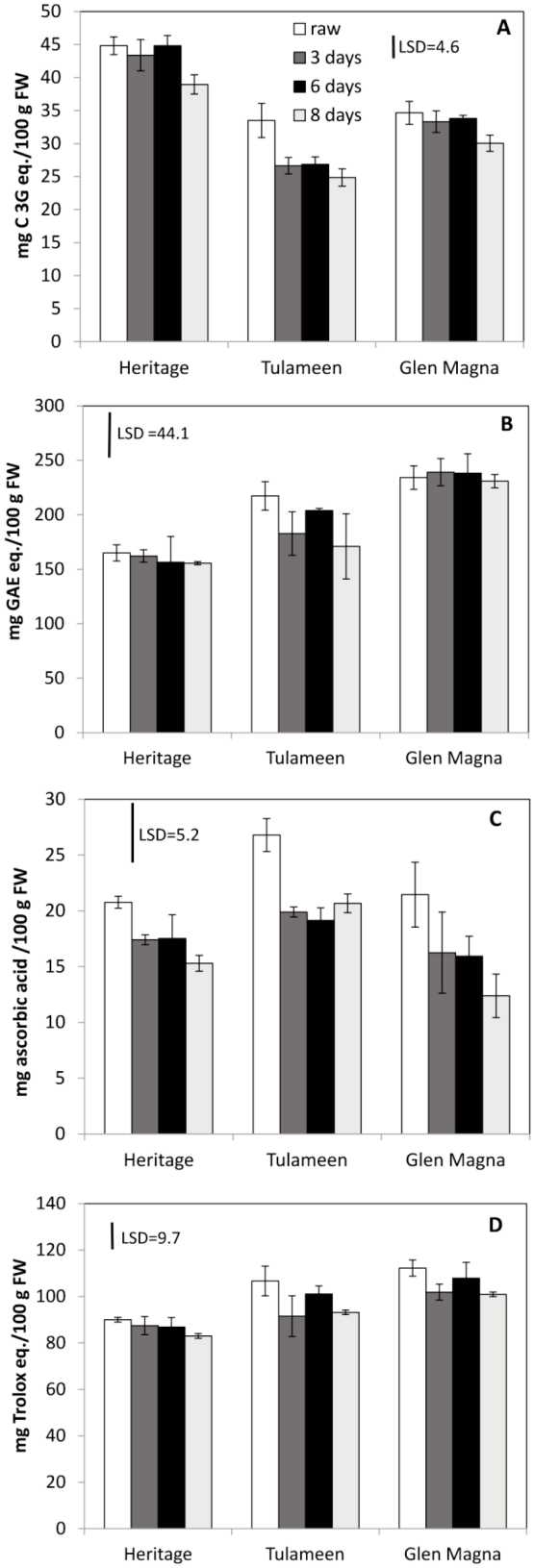

Fig. 1 - Total content (mean±standard error) of anthocyanins (A), phenols (B), Ascorbic acid (C) and total scavenging activity (D) measured in ready-to-eat samples from three raspberry cultivars, at harvest (raw) and after 3-6-8 days of shel-life at 3°C. The least significant distance (LSD) for P≤0.05 is indicated on every single graph.

colour parameters a* and b*, anthocyanins, acidity and SSC, opposite to scavenging activity, polyphenols content, L* and pH. PC2 (23.7%) was positively related to ascorbic acid, hue and SSC. In the biplot PC1 versus PC2 the three cultivars are very well distinguished. Heritage samples showed positive PC1 scores, linked to their high values of anthocyanins, acidity, a* and b* values. On the other side of the plot, 'Glen Magna' samples were characterized by low values on PC1 , due to their high DPPH scavenging activity and polyphenols content, L* parameter and pH values. All the 'Tulameen' samples had positive scores on PC2, linked to high ascorbic acid, hue and SSC. All the raw samples showed positive PC2 scores, being characterized by higher ascorbic acid content and more brilliant red colour (higher hue value). These values decreased markedly during shelf-life and, after only 3 days, all the samples showed lower PC2 scores.

4. Discussion and Conclusions

The weight loss can be an important problem during the postharvest of raspberry fruit (Haffner *et al*., 2002) however, in this experiment it always remained far below the limit of marketability (6%) (Haffner *et al*., 2002) and all fruit showed a good hydration state at the end of the shelf-life.

As it is visually shown in the PCA analysis, the raspberry cultivars were characterized by different quality parameters: in particular 'Heritage' by a higher anthocyanin content, acidity, a* and b* value, while 'Glen Magna' by a higher phenolic content and scavenging activity and 'Tulameen' by a higher ascorbic acid content.

At harvest SSC of raw fruit of 'Heritage' and 'Tulameen' was lower than those reported by the literature (Çekic and Özgen, 2010; Granelli *et al.*, 2010; Krüger *et al.*, 2011;) while the values of 'Glen Magna' were similar to those described by Mazur *et al.* (2014 a). The decrease in soluble solids content showed by all the cultivars during storage, as well as the reduction in acidity, was probably due to the normal respiration activity of the fruit (Giuggioli *et al.*, 2015). In any case, SSC was still at a good level at the end of shelf-life, showing a fairly good potential for these raspberry cultivars as a ready-to-eat product. It is well established, in fact, that SSC value is generally correlated with desirable flavor quality (Kader, 1997). The decrease of total acidity and the drop of the pH over time were also observed in other fresh-cut

products (Wright and Kader, 1997).

Tissue softening is a very serious problem and it often represents a limiting factor for many ready-to-eat products. Since consumers tend to want firm fruit, it is essential to evaluate fruit firmness in order to better assess the global quality of the product during shelf-life. Firmness of fresh-cut fruits has been widely reviewed but no data about this kind of ready-to-eat whole product are available. Besides, raspberry fruit is very fragile and the firmness is difficult to measure. The decrease in the firmness showed after only 3 days, even though the fruit tissues weren't injured by cutting, could be due to the necessary washing and sanitizing step. This operation consisted of a first dip in chlorinated water, to reduce the microbial loads on the fruit surface, and a second rapid dip in tap water, to eliminate residual chlorine and keep the sensorial properties of the untreated fruit. Moreover, the gentle drying of wet surfaces by adsorbent paper, a crucial step to remove the excess water, could cause mechanical damage to such delicate raspberries structures even though it is carried out carefully.

The stress caused from the washing step might also have affected the fruit color. By the way, the darkening of the fruit, which could affect the consumer choice, is reported by other authors (Haffner *et al.*, 2002; Giovanelli *et al.*, 2014) for fresh or packed raspberries stored in normal atmosphere at low temperature. In addition, Haffner *et al.* (2002) showed that storage in controlled atmosphere kept the berries more attractive than when stored in normal atmospheres.

Raspberry red colour is usually related to their anthocyanin composition (García-Viguera *et al.*, 1998). The higher initial anthocyanin content (Fig. 1A) and the higher value of the red parameter a* (Table 2) observed in 'Heritage', if compared to the other two cultivar, would confirm this positive correlation. The slight decrease of anthocyanin showed by the packed raspberry fruit during storage is in accordance with the results of Nunes *et al.* (2005) on strawberry stored at 1°C for 8 days. The author asserted that pigment degradation may be caused by the increased polyphenols oxydase activity as a result of physiological stress due to water loss during storage. Conversely, other authors reported an increase of the total anthocyanin content both in raspberry packed and stored at 4°C for 7 days (Giovanelli *et al.*, 2014) and in fresh fruit stored at 20°C for 1 day (Krüger *et al.,* 2011).

The total phenolics content (TPC) (Fig. 1B) of raw

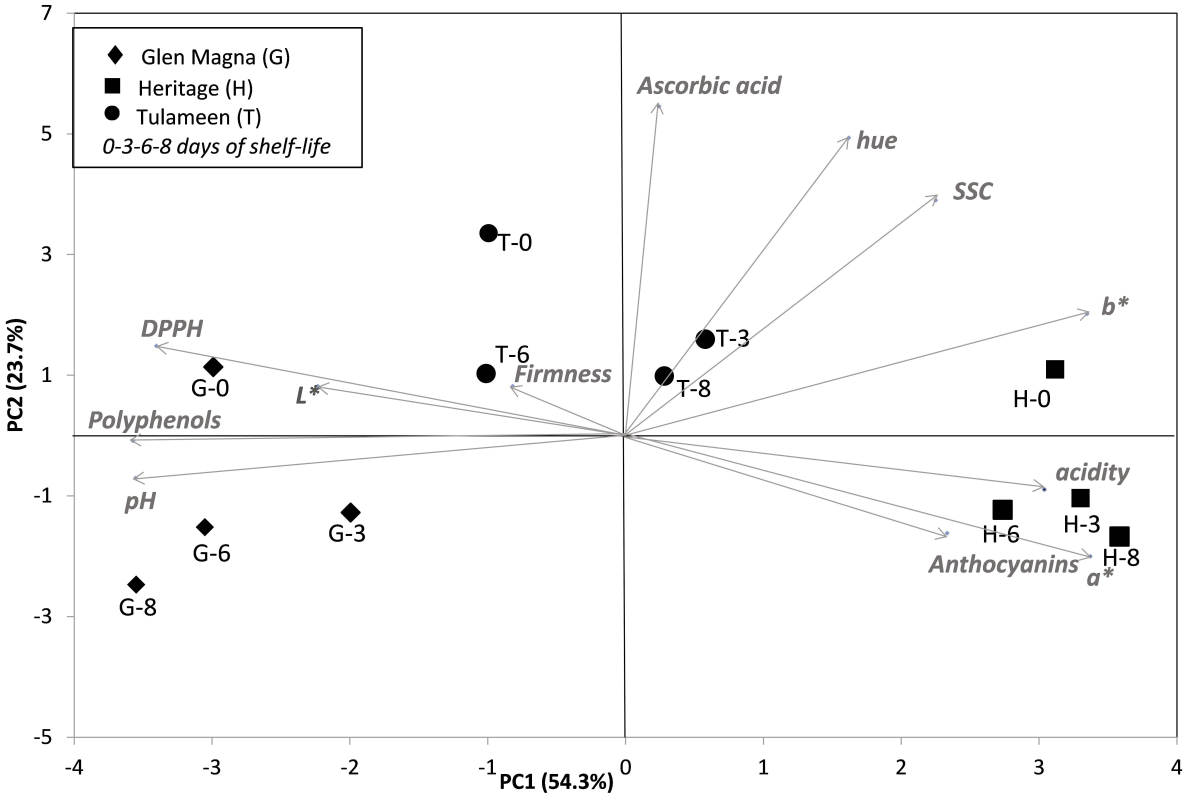

Fig. 2 - PCA biplot of quality and nutritional parameters during shelf-life of ready-to-eat raspberry fruit from the cultvars Heritage (H), Tulameen (T) and Glen Magna (G) after 0(raw)-3-6-8 days of shelf-life at 3°C (SSC=soluble solids content; L*, a*, b*, hue=colour parameters; DPPH=Scavenging activity).

'Heritage' reflected the findings of Cekic and Özgen (2010) whereas de Ancos *et al.* (2000) and Granelli *et al.* (2010) reported lower TPC values for this cultivar. TPC observed in the cv. Tulameen (Cekic and Özgen, 2010; Kruger *et al.,* 2011) and 'Glen Magna' (Mazur *et al.*, 2014 a) were in accordance with those reported in the literature. The stability of phenolics showed by the analyzed cultivar during shelf-life was also reported by Giovanelli *et al.* (2014) for fresh fruit and by de Ancos *et al.* (2000) for another type of processed raspberries product such as long-term frozen stored.

Ascorbic acid is one of the most important parameters used to control plant-derived food quality. Reduced temperature after harvest is considered to be important for its content in fruit (Krüger *et al.*, 2011). The decrease trend of the ascorbic acid of the analyzed cultivars during storage fully reflects the findings of de Ancos *et al.* (1999) and Krüger *et al.* (2011). Our results are, instead, not in accordance with Kalt *et al.* (1999) and Haffner *et al.* (2002) who found that AA level of raspberries, not washed and kept in normal atmosphere, was not reduced by several days of cold storage. A reasonable explanation for this discrepancy may be found in the washing step involved in the ready-to-eat processing: the hypochlorite contained in the sanitizing solution used could have oxidized the ascorbic acid, as reported by Bielski (1982).

It is well known that the scavenging activity of raspberry fruit is due to their high content of anthocyanins, phenolics and ascorbic acid (Krüger *et al.*, 2011). A significant correlation (R^2 = 0.857) was observed between the DPPH values and those of total phenol whereas the scavenging activity were not significantly correlated with total anthocyanins nor ascorbic acid. Our results, although lower than those found by Benvenuti *et al.* (2004), confirmed that the radical scavenging is related mainly to total phenol content.

The results of this study indicated the loss of firmness as the more problematic aspect of the ready-to-eat raspberries, followed by a less important change of the fruit colour from light red to dark red. The variations of chemical-physical parameters such as soluble solids content, pH and total titratable acidity, did not compromise the product quality. The processing (washing and packing) and the cold storage affected only slightly the nutraceutical profile, except for ascorbic acid, therefore the ready-to-eat raspberries could be considered a good source of compounds with potential health benefits. Some handling difficulties were highlighted during the processing steps of the samples (washing, drying and packing) due to the high fragility of fruit which caused a high percentage of waste. The hollow structure of raspberries made the drying step particularly difficult, however this is necessary as the residual water inside the fruit could significantly compromise the shelf-life of the product. In order to make this product more attractive to the consumers the loss of firmness and the darkening should be limited. For this purpose an alternative washing system like a fine spray and a packing in modified atmosphere could be adopted.

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