Effect of modified atmosphere on polyphenols during storage of pasteurised strawberry purées

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ARTICLE INFO

ABSTRACT

The minimum loss of processed fruit nutritional quality must been guaranteed during storage and the atmosphere can be a limiting step.

Strawberry purées flushed with gas mixtures: 10 kPa O_2 + 90 kPa N_2, 100 kPa N_2 and air (78 kPa N_2 + 21 kPa O_2 + 0.03 kPa CO_2) were stored for 90 days at 4 and 23 °C and revealed no effect in total antioxidant activity and in total phenolic content. The compounds (+)-catechin, (−)-epicatechin and quercetin-3-rutinoside were not affected by the atmospheres for both temperatures and ellagic acid was the exception within strawberry phytochemicals, where its concentration was higher for samples stored in air.

Total anthocyanin content was better preserved when strawberry purée was stored in 100 kPa N_2 at 4 and 23 °C, at which temperatures their levels decreased 24 and 77%, respectively. At 4 °C cyanidin-3-glucoside presented no significant differences between atmospheres. Pelargonidin-3-glucoside and pelargonidin-3-rutinoside decreased both 27% for 100 kPa N_2 and 45% for 10 kPa O_2 and air. All the individual anthocyanins were not affected by the atmospheres when stored at 23 °C.

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1. Introduction

Modified atmosphere has been used as a way to better control undesirable changes promoted by oxidative reactions. Modified atmosphere can be defined as one that is created by altering the normal composition of air (78 kPa nitrogen, 21 kPa oxygen, 0.03 kPa carbon dioxide and traces of noble gases) to provide an adequate, although not strictly controlled, atmosphere for increasing the storage period and quality of food (Farber et al., 2003).

Industrially processed fruit are subjected to different unit operations that can affect their structure, favouring the occurrence of chemical reactions (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007; García-Torres, Ponagandla, Rouseff, Goodrich-Schneider, & Reyes-De-Corcuera, 2009). During these procedures cells compartmentation is lost, cell walls disrupted and air is incorporated into the purées.

Metabolites and enzymes that are compartmentalized are mixed, producing chemical and biochemical reactions, some of them promoted by oxygen present in air leading to oxidation reactions that often result in browning, changes in aroma, and loss of nutritional value (García-Torres et al., 2009).

The nutritional quality of strawberry is related primarily to their richness in polyphenols (Klopotek, Otto, & Bohm, 2005). Diversity and content of polyphenols in processed strawberry may decrease during storage, depending on conditions such as time, temperature and oxygen content (Alwazeer, Delbeau, Divies, & Cachon, 2003; Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012; Kalt, McDonald, & Donner, 2000).

Studies concerning adverse effects of dissolved oxygen on the quality attributes of fruit juices have been reported by many researchers including studies about ascorbic acid degradation by oxidation reactions (Aguilar-Rosas et al., 2007; Solomon, Svanberg, & Sahlström, 1995; Zerdin, Rooney, & Vermuë, 2003).

In fruit processing, usually modification of an atmosphere involves a reduction of oxygen or an increase of carbon dioxide or nitrogen concentrations. The lower content oxygen has reported to

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slow down spoilage microorganisms and nitrogen is used to displace oxygen in order to retard aerobic spoilage and oxidative deterioration (Erkmen, 2012).

The atmospheres choice for this study was dependent on prevention of oxidative deterioration. Air was used as control, 100 kPa N_2 was selected to represent the total absence of oxygen and a mild O_2 concentration of 10 kPa was selected. To minimize fruit nutritional quality losses, the atmosphere along storage must be controlled beyond processing. Given the lack of information about chemical changes occurring in fruit after processing, this study gives an additional survey to the established information.

The main purpose of this work was to evaluate phytochemicals concentration during 90 days-storage of pasteurised strawberry purée under three atmospheres (air, 100 kPa N_2 and 10 kPa O_2 + 90 kPa N_2) and at two storage temperatures (4 and 23 °C) for future recommendations to improve polyphenols preservation of fruit processed.

2. Materials and methods

2.1. Chemicals

Methanol, formic acid (Sigma–Aldrich, Sintra, Portugal) and hydrochloric acid (Merck, Oeiras, Portugal) were all of HPLC grade. Reagents used for determinations of total phenolic compounds, antioxidant activity and total anthocyanins were 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium chloride, potassium sorbate, sodium carbonate, sodium acetate (Sigma–Aldrich, Sintra, Portugal), Folin-Ciocalteu's reagent and potassium persulfate (Merck, Oeiras, Portugal). Standards used for calibration curves were ascorbic acid, gallic acid, (+)-catechin, (−)-epicatechin, ellagic acid, quercetin-3-rutinoside (Sigma–Aldrich, Sintra, Portugal), cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside (Extrasynthése, Lyon, France).

2.2. Strawberry purée treatments

Individually quick frozen (IQF) strawberries [*Fragaria × ananassa* Duch. cv. 'Camarosa'] were blended to obtain purée. Strawberry purée was mixed with 0.25 g/L of natamycin and 1.34 g/L of potassium sorbate and 60 g were poured in serum type reaction glass vials of 100 mL. Vials with the purée were pasteurised at 90 °C and the temperature at the centre of the vial, monitored with an HD 8802 thermometer (Delta OHM, Padova, Italy), reached 89.5 °C ± 2.2 °C (mean ± SD, $n = 20$) after 15 min and remained at that temperature for 5 min thereafter. The vials were then cooled to room temperature in ice during 5 min. In a flow hood under aseptic conditions the vials with sample were flushed with a continuous flow at 40 kPa of humidified air (control), 100 kPa N_2 and 10 kPa O_2 + 90 kPa N_2 and at the end of 3 min vials were crimped with aluminium seal with Teflon faced butyl septa. Vials were stored in dark for 90 days at room (*ca.* 23 °C) and cold temperature (4 °C).

Samples to perform extraction were taken in triplicates after 24 h and during storage (7, 14, 30, 60 and 90 days) for each atmosphere and temperature. The headspace gases were checked regularly with a PBI Dansensor CheckMate 3 (Dansensor, Ringsted, Denmark) throughout all storage period where a needle was plunged into the vials through septa to determine oxygen, carbon dioxide and nitrogen concentrations inside the package.

2.3. Microbiological analysis

The samples (10 mL) were homogenized in a stomacher (Model 400 Circulator, Seward, Norfolk, England) with peptone (Sigma, Sintra, Portugal) water (90 mL), serially diluted and plated on Plate

Count Agar (PCA, Biokar Diagnostics, Solabia, France) and incubated at 30 °C during 24 h. Total yeasts and molds were also determined for the raw samples using Potato Dextrose Agar - PDA (Biokar Diagnostics, Solabia, France). The plates were incubated for 2 days at 30 °C. In both cases, colonies were enumerated and total viable cells (cfu/g) determined.

2.4. Extracts preparation

Polyphenols were extracted by maceration of 2.5 g of strawberry purée with 25 mL of aqueous methanolic solvent at 800 mL/L using an ultra-turrax (IKA T18, Wilmington, USA) operated at 24,000 rpm for 60 s. The extract was centrifuged at 4000×g, 4 °C for 10 min and the supernatant filtered through a 0.45-μm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and used for total activities measurement. A 15-mL aliquot of the supernatant was concentrated in an RVC 2-18 speed-vacuum evaporator (Christ, Osterode, Germany) at 30 °C and the residue dissolved in 4 mL of methanol and analysed by HPLC-DAD.

The same procedure was used for anthocyanins extraction, except that acidified methanol was used (methanol:water:HCl 12 N:800 mL: 150 mL: 50 mL).

2.5. Total antioxidant activity

The ABTS radical scavenging activity was measured in the non-acidified methanolic extracts using the method described by (Giao et al., 2007). Ascorbic acid was used as a standard to prepare a calibration curve in the range of 0.02–0.50 g/L.

2.6. Analysis of total phenolic compounds

The concentration of total phenolic compounds was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton & Rossi, 1965). Quantification was done at 750 nm (UV mini 1240, Shimadzu, Tokyo, Japan) with gallic acid as standard in the range 0.015–1.00 g/L.

2.7. Analysis of total anthocyanins

The concentration of total anthocyanins was determined spectrophotometrically by the pH-differential method described by Lee, Durst, and Wrolstad (2005), with some modifications. Quantification was done at 515 and 700 nm at pH 1.0 and pH 4.5.

Results were expressed in accordance with Eq. (1) and converted to milligram of pelargonidin-3-glucoside per gram of biomass.

$$A = (A_{515} - A_{700})_{pH1.0} - (A_{515} - A_{700})_{pH4.5} \quad (1)$$

c (mg/l) = (A × molecular weight × dilution factor × 1000)/(εl)

Molecular weight of pelargonidin-3-glucoside is 433.0 g/mol and ε is 22,400 mol^{-1}

2.8. Measurements of polyphenols

Samples were analysed on an HPLC-DAD (Waters Series 600, Mildford, MA, USA). A Symmetry® C-18 column, 250 × 4.6 mm, i.d. 5 μm and 12.5 nm pore size with a guard column (Waters), was used and solvents elution consisted of solvent A – methanol/water/formic acid (92.5:5:2.5 v/v/v) and solvent B – methanol/water (94:6 v/v). For methanolic extracts, the linear gradient system starts on 0 and goes to 30% B in 40 min, 30–50% B in 20 min and from 50 to 0% B in 10 min. For anthocyanins a linear gradient starts at 0 and

goes to 30% B in 10 min, from 30 to 50% B in 15 min and finally to 0% B in 5 min and kept at 0% B during 5 min. The injection volume was 50 μL with flow rate of 0.75 ml/min and a column temperature maintained at 25 °C.

Absorbance was measured at 280 nm ((+)-catechin and (−)-epicatechin), 350 nm (ellagic acid and quercetin-3-rutinoside) and 510 nm (cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside) with a diode array detector (Waters, Mildford, MA, USA). Quantifications were made by the use of pure standards and expressed as micrograms per gram of fresh biomass.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows. Normality of data distribution was tested by Kolmogorov−Smirnov method.

Statistical significance of differences among group means was evaluated by two-way analysis of variance with Bonferroni post hoc test at the $P < 0.05$ level.

3. Results and discussion

3.1. Headspace gas composition

The variation of headspace gas partial percentages during the storage of pasteurised strawberry purée, packaged under different modified atmospheres (100 kPa N_2, 10 kPa O_2 + 90 kPa N_2, and air) for 90 days storage at 4 and 23 °C is presented on Fig. 1.

Oxygen levels in the headspace vials were stable during storage under the conditions tested. Significant ($P < 0.05$) differences were detected, between both temperatures (4 and 23 °C), in samples stored under air and 10 kPa O_2 + 90 kPa N_2. Under air at the end of 90 days at 23 °C the oxygen level decreased from 19% to 18% while at 4 °C it was stable maintaining 20% oxygen. In 10 kPa O_2 + 90 kPa N_2, there was a decrease from 10 kPa O_2 to 7.8 kPa at 23 °C, while at 4 °C storage the levels of 10.0 kPa O_2 was maintained (Fig. 1). The oxygen levels decrease revealed that oxygen was removed from the headspace and probably by dissolution in the matrix or consumed by oxidation reactions.

Carbon dioxide was only added to samples stored under air, but in the other atmospheres carbon dioxide was also detected inside the flasks in concentrations lower than 3 kPa. Besides that, there was a significant difference ($P < 0.05$) for both storage temperatures between days 7 and 90, where higher concentrations were detected in samples stored at 23 °C (Fig. 1). Given that gases were added to the flask headspace, the increase in carbon dioxide observed at 23 °C could be a result of CO_2 transference from the purée air spaces to the flask headspace.

Nitrogen was stable during storage without differences between both storage temperatures (Fig. 1), and the nitrogen levels reach stability at day 15 of storage. It was observed that 100 kPa N_2 decreased from 100 to 95 kPa and then returns to 99 kPa. Under air, atmosphere reaches a stable value of 78 kPa at day 15 while storage under 10 kPa + 90 kPa at day zero presented 95 kPa, which decreased to 89 kPa. Nitrogen is an inert gas with low solubility in water and fat (Erkmen, 2012). These results revealed that about 15 days were necessary to reach equilibrium of nitrogen content in flask headspace.

3.2. Total antioxidant activity, total phenolics and total anthocyanins

Strawberry IQF was analysed for the initial antioxidant activity and content of total phenolic and total anthocyanins. The antioxidant activity determined by the ABTS was of 1.2 ± 0.1 mg of ascorbic
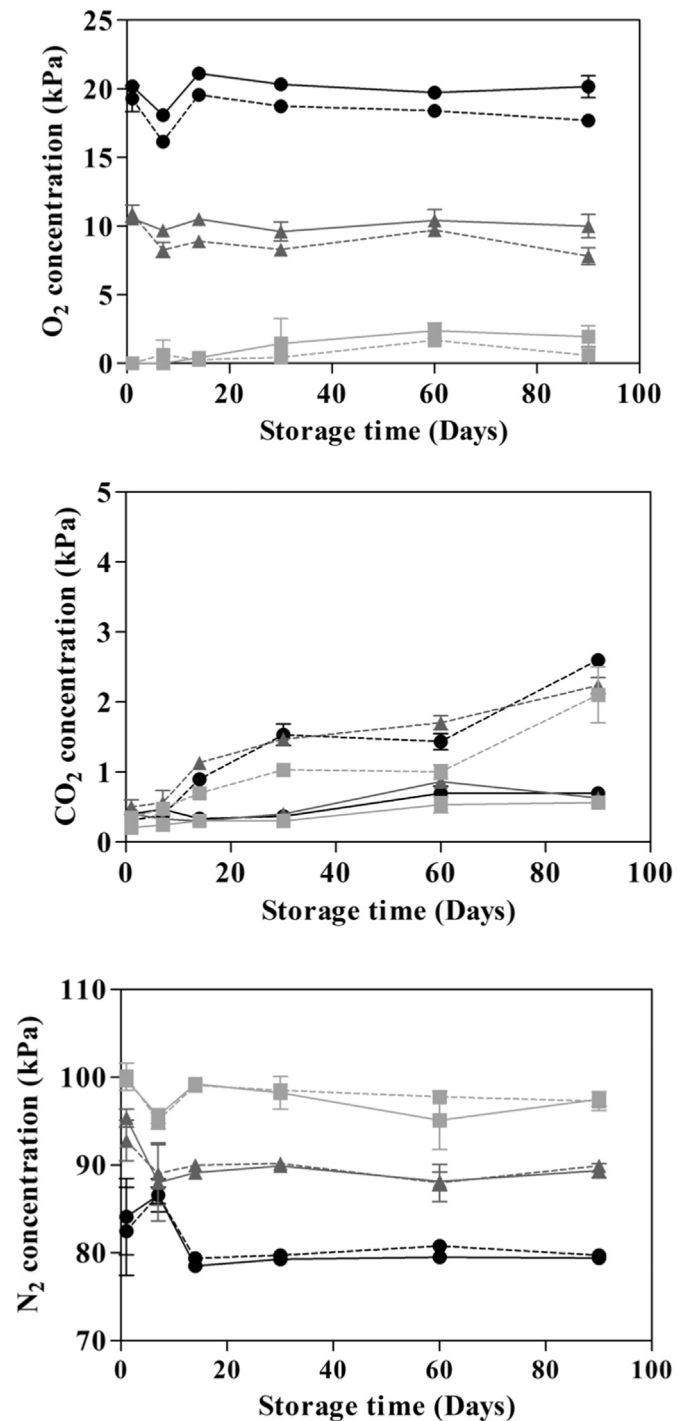

Fig. 1. Concentration of oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) for each modified atmosphere during storage of pasteurized strawberry purée stored at 4 °C (—) and 23 °C (⋯) for 90 days. Results are expressed as the average ± SD ($n = 3$). The different atmosphere conditions are represented as ●: Air. ■: 100 kPa N_2. ▲: 10 kPa PaO_2 + 90 kPa N_2.

acid/g biomass, the total phenolic content was 1.3 ± 0.1 mg of gallic acid/g biomass and total anthocyanins was 0.4 ± 0.0 mg of pelargonidin-3-glucoside/g biomass. Very similar values were obtained by Padula et al. (2013) for camarosa fresh strawberries, with a content of 1.4 and 0.4 mg/g biomass, respectively for total phenols and total anthocyanins. However, results obtained for antioxidant activity (3.5 mg of trolox/g fw) were higher because the

determinations were made with a different standard as reference and with a different method. The DPPH method combines reactions of electron transfer with hydrogen atom transfer while ABTS only determine a single electron transfer reaction (Pérez-Jiménez et al., 2008).

Total antioxidant activity of strawberry purée was not affected during 90-day storage under different atmospheric conditions (air, 100 kPa N_2 and 10 kPa O_2 + 90 kPa N_2) (Tables 1 and 2). On the other hand, it was strongly affected by storage time and temperature, where at 4 °C decreased 35% for all atmospheric conditions and at 23 °C the reduction was of 50, 53 and 58% for 100 kPa N_2, 10 kPa O_2 + 90 kPa N_2 and air, respectively.

As reported by Fernandes et al. (2013) and Georgé, Brat, Alter, and Amiot (2005) the Folin−Ciocalteu's assay is one of the oldest and accepted method designed to evaluate the total phenolic content in vegetal matrices. The method presents the major disadvantage of overestimating the polyphenol content, since the Folin−Ciocalteu reagent reacts with many reducing substances such as vitamin C, sugar, amino acids, etc (Everette et al., 2010). The total phenolic content of the strawberry samples was not affected by modified atmosphere (Tables 1 and 2). Under refrigerated storage (4 °C) total phenolic content decreased 38% for air and 31% for 100 kPa N_2 and 10 kPa O_2+90 kPa N_2. At 23 °C phenolic content decreased 40% for 100 kPa N_2, 53 and 57% for 10 kPa O_2+90 kPa N_2 and air, respectively.

Studies reported changes in antioxidant capacity promoted by modified atmosphere such as Kalt et al. (2000). They described a decrease of 30 and 46% in total antioxidant activity and total phenolic content in blueberry juice stored for 6 h in half-full vessels with air. Piljac-Žegarac, Valek, Martinez, and Belščak (2009) reported, antioxidant capacity decrease in strawberry, cranberry, blueberry and pomegranate juices stored under refrigeration for 29 days.

Storage under air, 10 kPa O_2 or 100 kPa N_2 was equivalent for antiradical capacity and total phenolics of strawberry purée. The best condition for storage was lower temperature (4 °C), since the

Table 1
Effect of modified atmosphere on antioxidant activity, total phenolic and total anthocyanin content of pasteurized strawberry purée stored at 4 °C for 90 days. Results are expressed as the average ± SD ($n = 3$) of concentration balance where C_t is the compound concentration at time t (mg/g biomass) and C_0 is the compound concentration at time 0.

Storage time (days)	Air	100 kPa N_2	10 kPa O_2 + 90 kPa N_2
Antioxidant activity [C_t/C_0]			
1	1.0 ± 0.0[a]	1.0 ± 0.1[a]	1.0 ± 0.0[a]
7	1.2 ± 0.2[a]	1.0 ± 0.1[a]	0.9 ± 0.1[b]
14	1.0 ± 0.0[ac]	0.9 ± 0.1[a]	1.0 ± 0.1[a]
30	0.8 ± 0.0[a]	0.8 ± 0.0[a]	0.7 ± 0.0[a]
60	0.7 ± 0.0[a]	0.7 ± 0.0[a]	0.7 ± 0.0[a]
90	0.7 ± 0.0[a]	0.7 ± 0.0[a]	0.6 ± 0.0[a]
Total phenolics [C_t/C_0]			
1	1.0 ± 0.2[a]	1.0 ± 0.1[a]	1.0 ± 0.0[a]
7	0.9 ± 0.0[a]	1.0 ± 0.0[a]	0.9 ± 0.0[a]
14	0.9 ± 0.0[a]	1.0 ± 0.0[a]	1.1 ± 0.1[a]
30	0.9 ± 0.0[a]	1.0 ± 0.0[a]	1.0 ± 0.0[a]
60	0.8 ± 0.0[a]	1.0 ± 0.1[a]	0.9 ± 0.1[b]
90	0.6 ± 0.0[a]	0.7 ± 0.0[a]	0.7 ± 0.0[a]
Total anthocyanins [C_t/C_0]			
1	1.0 ± 0.0[a]	1.0 ± 0.0[a]	1.0 ± 0.0[a]
7	1.0 ± 0.0[a]	1.0 ± 0.0[a]	0.9 ± 0.0[a]
14	1.0 ± 0.0[ab]	1.0 ± 0.0[a]	0.9 ± 0.0[b]
30	0.9 ± 0.0[ab]	0.9 ± 0.0[b]	1.0 ± 0.0[a]
60	0.8 ± 0.0[b]	0.9 ± 0.0[ac]	0.8 ± 0.0[c]
90	0.7 ± 0.0[b]	0.8 ± 0.0[ac]	0.7 ± 0.0[c]

[a,b,c]Different letters represents significant ($P < 0.05$) differences between packaging atmospheres for each storage day.

Table 2
Effect of modified atmosphere on antioxidant activity, total phenolic and total anthocyanin content of pasteurized strawberry purée stored at 23 °C for 90 days. Results are expressed as the average ± SD ($n = 3$) of concentration balance where C_t is the compound concentration at time t (mg/g biomass) and C_0 is the compound concentration at time 0.

Storage time (days)	Air	100 kPa N_2	10 kPa O_2 + 90 kPa N_2
Antioxidant activity [C_t/C_0]			
1	1.0 ± 0.1[a]	1.0 ± 0.0[a]	1.0 ± 0.1[a]
7	0.8 ± 0.0[b]	1.0 ± 0.2[a]	0.9 ± 0.0[ab]
14	0.7 ± 0.0[b]	0.9 ± 0.0[a]	0.8 ± 0.0[ab]
30	0.6 ± 0.0[b]	0.7 ± 0.0[a]	0.6 ± 0.0[a]
60	0.4 ± 0.0[b]	0.6 ± 0.1[a]	0.6 ± 0.1[a]
90	0.4 ± 0.0[b]	0.5 ± 0.1[a]	0.5 ± 0.0[a]
Total phenolics [C_t/C_0]			
1	1.0 ± 0.1[a]	1.0 ± 0.1[a]	1.0 ± 0.0[a]
7	0.9 ± 0.0[a]	1.0 ± 0.0[a]	0.9 ± 0.1[a]
14	0.8 ± 0.1[a]	1.0 ± 0.0[a]	0.9 ± 0.1[a]
30	0.6 ± 0.1[b]	0.9 ± 0.0[a]	0.8 ± 0.0[b]
60	0.7 ± 0.1[ab]	0.9 ± 0.1[a]	0.6 ± 0.1[b]
90	0.4 ± 0.0[b]	0.6 ± 0.0[b]	0.5 ± 0.0[b]
Total anthocyanins [C_t/C_0]			
1	1.0 ± 0.0[a]	1.0 ± 0.0[a]	1.0 ± 0.0[a]
7	0.8 ± 0.0[a]	0.9 ± 0.0[a]	0.9 ± 0.1[a]
14	0.6 ± 0.0[b]	0.8 ± 0.0[a]	0.7 ± 0.0[b]
30	0.5 ± 0.0[b]	0.7 ± 0.0[a]	0.5 ± 0.0[b]
60	0.2 ± 0.0[c]	0.5 ± 0.1[a]	0.4 ± 0.2[bc]
90	0.1 ± 0.0[c]	0.2 ± 0.1[a]	0.2 ± 0.0[c]

[a,b,c]Different letters represents significant ($P < 0.05$) differences between packaging atmospheres for each storage day.

observed decreases were all inferior to 40%, in detriment of room temperature.

Total anthocyanins content also changed exhibiting a similar pattern as observed for total antioxidant activity and total phenolic content during storage in response to different atmospheric treatments. At 4 °C, after 90 days, total anthocyanins content decreased 35% in air, which was significantly different ($P < 0.05$) from samples stored at 100 kPa N_2 and 10 kPa O_2 + 90 kPa N_2, which decreased 24 and 29%, respectively. At 23 °C samples stored under 100 kPa N_2 decreased 77%, which was significantly ($P < 0.05$) lower than 10 kPa O_2 + 90 kPa N_2 and air, where it decreased 82 and 86%, respectively (Tables 1 and 2).

Lower oxygen content as well refrigerated temperature (4 °C) were the better conditions to achieve strawberry purée with higher anthocyanins content. Oxygen has been reported to be an important factor in destabilizing anthocyanins in processed products such as strawberry juices (Francis & Markakis, 1989; Nebesky, Esselen, Mc Connell, & Fellers, 1949). Clydesdale, Main, Francis, and Damon (1978) reported an increased stability of grape pigments in a dry beverage mix by flushing with nitrogen.

Shikov, Kammerer, Mihalev, Mollov, and Carle (2008) observed that lowering storage temperature from 20 to 4 °C in strawberry purée was the most effective measure to improve anthocyanins retention over time. Storage of strawberry jams, during 24 weeks at 20 and 4 °C, presented a significantly decline in total and individual anthocyanin contents (Shikov, Kammerer, Mihalev, Mollov, & Carle, 2012). Buchweitz, Carle, and Kammerer (2012) observed a 10% decrease in anthocyanins of strawberry stored 18 weeks at 20 °C in the dark.

### 3.3. Individual phytochemicals content during storage

High-performance liquid chromatography (HPLC) was used to separate and determine individual phenolic compounds in strawberry purée samples.

The (+)-catechin presented an initial concentration, on IQF samples, of 43.9 ± 4.1 µg/g similar to the values of 58.6 µg/g

obtained by Padula et al. (2013) in fresh strawberry. At 4 °C, (+)-catechin presented high stability during 90 days-storage (Fig. 2A), contrarily to samples stored at 23 °C where air led to a significant ($P < 0.05$) decrease of 45% after 30 days of storage. In 10 kPa O_2 + 90 kPa N_2 and 100 kPa N_2 the observed decrease was only 16% (Fig. 3A). (−)-Epicatechin did not shown differences between the atmospheres for both temperatures. At 4 °C, (−)-epicatechin concentration decreased 9% under 100 kPa N_2, 4% for 10 kPa O_2+90 kPa N_2 and 10% for air at the end of storage. At 23 °C it decreased 61% under anerobiosis (100 kPa N_2),

followed by 45% for 10 kPa O_2 + 90 kPa N_2 and 30% for air (Figs. 2B and 3B).

Polyphenols from green and black teas show a pronounced oxygen consumption leading to autoxidation of compounds like epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Roginsky & Alegria, 2005). Similar behaviour is reported for autoxidation of tea catechins, which showed to be highly endothermic, so elevation of temperature stimulates oxidation reactions (Roginsky & Alegria, 2005; Roginsky & Barsukova, 2000).
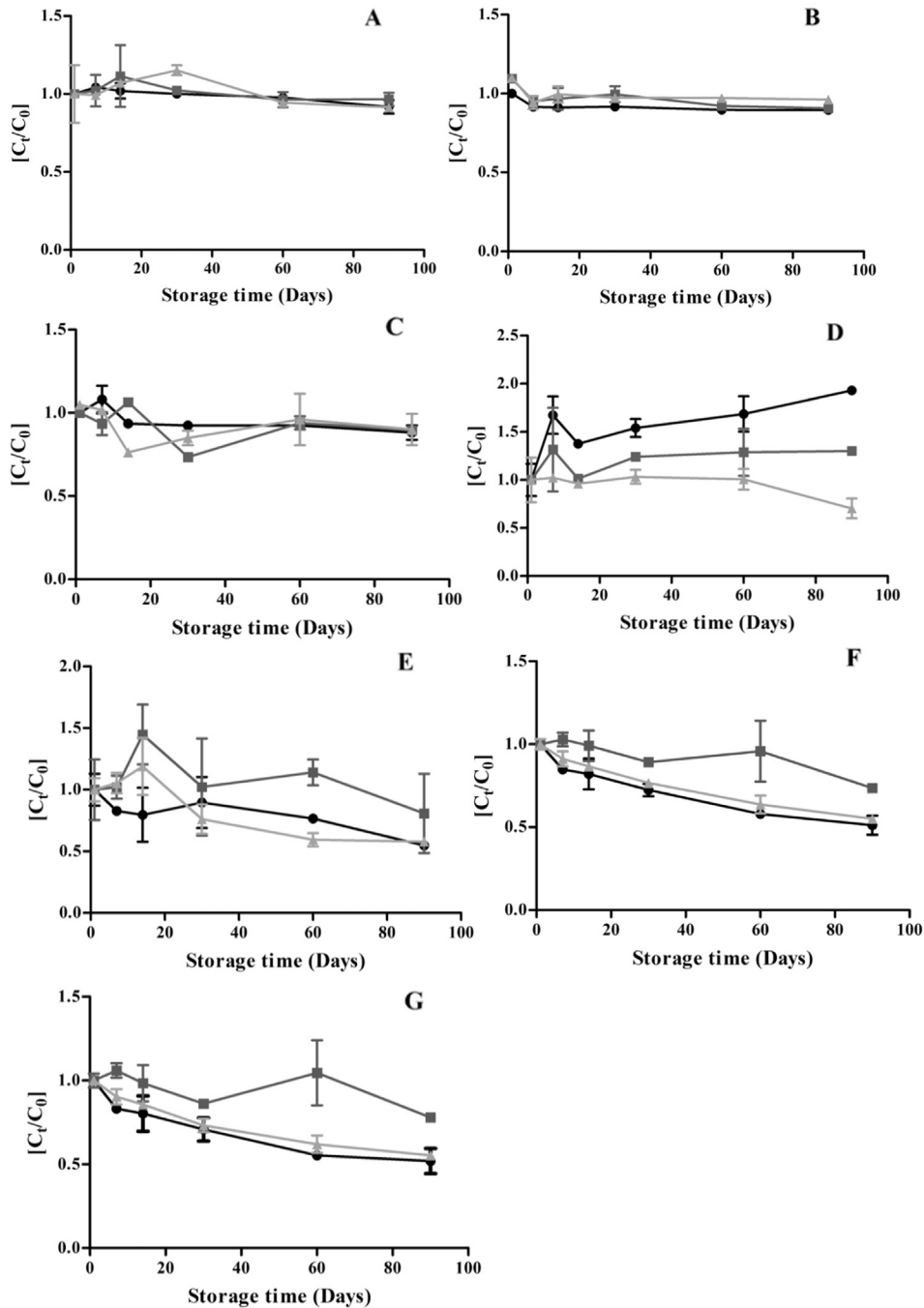
Fig. 2. Effect of modified atmosphere on the concentration of individual phenolic compounds during storage of pasteurized strawberry purée at 4 °C for 90 days. Results are expressed as the average ± SD ($n = 3$) of concentration balance where C_t is the compound concentration at time t (μg/g fw), C_0 is the compound concentration at time 0. The different atmosphere conditions are represented as ●: Air. ■: 100 kPa N_2. ▲: 10 kPa PaO_2 + 90 kPa N_2. (A: (+)-catechin; B: (−)-epicatechin; C: quercetin-3-rutinoside; D: ellagic acid; E: cyanidin-3-glucoside; F: pelargonidin-3-glucoside; G: pelargonidin-3-rutinoside).
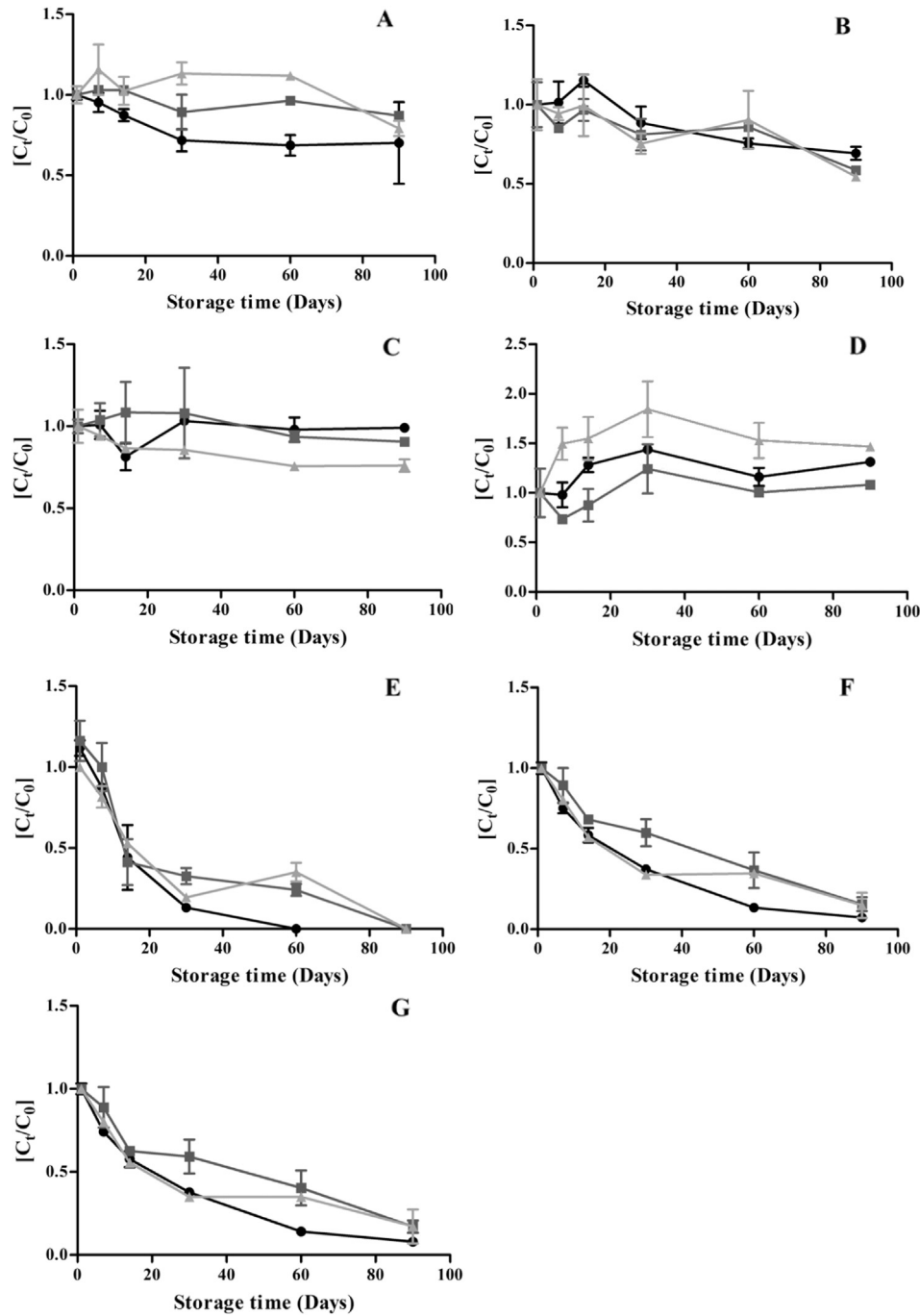
Fig. 3. Effect of modified atmosphere on the concentration of individual phenolic compounds during storage of pasteurized strawberry purée at 23 °C for 90 days. Results are expressed as the average ± SD ($n = 3$) of concentration balance where C_t is the compound concentration at time t (µg/g fw), C_0 is the compound concentration at time 0. The different atmosphere conditions are represented as ●: Air. ■: 100 kPa N_2. ▲: 10 kPa PaO_2 + 90 kPa N_2 (A: (+)-catechin; B: (−)-epicatechin; C: quercetin-3-rutinoside; D: ellagic acid; E: cyanidin-3-glucoside; F: pelargonidin-3-glucoside; G: pelargonidin-3-rutinoside).

Cheynier (2005) reported that flavanols can form dimeric adducts with anthocyanins by two types of chemical reactions. One involves cleavage of the tannin interflavanic linkage, followed by nucleophilic addition of the anthocyanin hemiketal to the carbocation. Second the anthocyanin and flavanol units can be linked by both carbon–carbon (C4–C8) and ether (C2–O–C7) bonds (A-type), formed by nucleophilic addition of the flavanol to the anthocyanin flavylium cation.

The anthocyanins identified in strawberry were cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutino

side and their concentration on IQF samples were of 5.0 ± 0.4, 298 ± 52 and 22.2 ± 0.4 µg/g biomass, respectively. Padula et al. (2013) describe, on camarosa strawberries, a content of 28.6, 179 and 22.9 µg/g respectively for cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside and pelargonidin-3-O-rutinoside. da Silva, Escribano-Bailón, Pérez Alonso, Rivas-Gonzalo, and Santos-Buelga (2007) reported a higher content in cyanidin-3-glucoside (25 µg/g) and pelargonidin-3-rutinoside (43 µg/g), while pelargonidin-3-glucoside content was very similar to our results, with 261 µg/g for fresh strawberry. However, these discrepancies are expected

due to the differences in climate conditions during production that inevitably affect the antioxidant compounds profile.

All the strawberry anthocyanins showed an increased degradation with the increase in oxygen levels. Cyanidin-3-glucoside decreased 20% when samples were stored under 100 kPa N_2 and 43% for storage under 10 kPa O_2 + 90 kPa N_2 and air at 4 °C (Fig. 2E). When samples were stored 90 days at 23 °C, cyanidin-3-glucoside decreased at levels below the detection limit for all the atmospheres tested (Fig. 3E).

The pelargonidin-3-glucoside content decreased 27% when strawberry purée was stored under 100 kPa N_2 at 4 °C. The other 2 atm tested induced a decrease of 45% (Fig. 2F). The 100 kPa N_2 allowed higher ($P < 0.05$) retention of pelargonidin-3-glucoside than air and 10 kPa O_2 + 90 kPa N_2. For 23 °C, pelargonidin-3-glucoside decrease 85% for 100 kPa N_2 while for 10 kPa O_2 + 90 kPa N_2 and air the decrease raised to 99.3% (Fig. 2F).

The 100 kPa N_2 allowed higher retention, at 4 °C, with a decrease of 23%. For the conditions of 10 kPa O_2 + 90 kPa N_2 and air pelargonidin-3-rutinoside decreased 45% (Fig. 2G). Room temperature (23 °C) induced higher reductions corresponding to 83% for 100 kPa N_2 and 99.3% for 10 kPa O_2 + 90 kPa N_2 and air, without differences between them (Fig. 3G).

The deleterious effects of molecular oxygen on anthocyanins have been observed by a number of researchers. Oxygen and temperature were the most specific accelerating factors in the degradation of anthocyanins (Nebesky et al., 1949). Others reports describe that the replacement of the oxygen atmosphere with nitrogen enhance the stability of cyanidin-3-diglucoside, a natural berry juice pigment (Daravingas & Cain, 1968). Oxygen incorporation into blueberry juice has a marked effect on monomeric anthocyanins with 76% loss after 6 h storage (Kalt et al., 2000) and it was considered a destabilizing agent for anthocyanins from processed products of blueberry juice (Francis & Markakis, 1989).

Oxygen may cause deleterious effects through a direct oxidative mechanism and/or trough indirect oxidation. Oxidized constituents of the medium, such as furfural-type compounds resultant from sugars degradation, are capable of reacting and/or condense with anthocyanins to form complex brown coloured compounds (Jackman, Yada, Tung, & Speers, 1987).

Aerobic oxidation of ascorbic acid promotes anthocyanins degradation (Sondheimer & Kertesz, 1953), as well condensation between ascorbic acid and anthocyanins as reported for strawberry juice (Markaris, Livingston, & Fellers, 1957).

The interaction between pelargonidin-3-glucoside, catechin and ascorbic acid is also observed, in model systems where ascorbic acid accelerates anthocyanin loss in samples stored under nitrogen as well as those stored under oxygenated conditions (Poei-Langston & Wrolstad, 1981; Sondheimer & Kertesz, 1953).

Quercetin-3-rutinoside concentration, at 4 °C, decreased 10% in all atmospheric conditions without significant differences along the storage period. At 23 °C quercetin-3-rutinoside decreased 1% when stored in air, 10% in 100 kPa N_2 and 24% in 10 kPa O_2 + 90 kPa N_2. The only significant differences ($P < 0.05$) were obtained for 10 kPa O_2 + 90 kPa N_2 and air between day 60 and 90 of storage.

Buchner, Krumbein, Rohn, and Kroh (2006) showed that presence of oxygen highly induces quercetin and rutin degradation, while the absence of oxygen has the opposite effects.

Quercetin of strawberry juices decreased progressively at 4 °C in darkness over 56 days (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2009).

Ellagic acid, is formed through the association of two molecules of gallic acid in the presence of oxygen and the dimer of gallic acid spontaneously forms ellagic acid when acidified (Tulyathan, Boulton, & Singleton, 1989). The initial concentration determined on IQF samples was of 12.7 ± 0.4 µg/g fw. It have been reported a great variety of concentrations for ellagic acid in camarosa strawberries, such as 6.1 µg/g (Van De Velde, Tarola, Güemes, & Pirovani, 2013), 22 µg/g (da Silva Pinto, Lajolo, & Genovese, 2008) and 1193 µg/g for fresh strawberries (Bojarska, Zadernowski, & Czaplicki, 2011). The storage at 4 °C revealed an increase of 92% for air, 30% decrease for 10 kPa O_2 + 90 kPa N_2 and 30% increase for 100 kPa N_2. The differences ($P < 0.05$) registered between three atmospheres occurred only between day 60 and 90 of storage. At 23 °C ellagic concentration increased 8, 47 and 31%, respectively for 100 kPa N_2, 10 kPa O_2 + 90 kPa N_2 and air. The concentration variations observed, at 23 °C in the end of 90 days, were not significantly different between them.

One of the reasons proposed for ellagic acid increase can be ellagitannins degradation caused by combination of thermal treatment and storage time (Aaby, Skrede, & Wrolstad, 2005; Häkkinen, Kärenlampi, Mykkänen, Heinonen, & Törrönen, 2000; Zafrilla et al., 2003).

The antioxidant capacity as well total phenolic content are associated with hydroxyl groups of a molecule and so depends on phenolic compound chemical structure, namely the number and arrangement of the hydroxylated groups (Rice-Evans, Miller, & Paganga, 1996; Sang et al., 2002).

However the individual compounds as a whole can reproduce a constant signal as observed for total activities, besides the variation observed in their concentration along storage.

Strawberry purée pH values were kept constant during storage starting with 3.8 ± 0.00 and ending at 3.5 ± 0.05 for all atmospheric conditions and temperatures tested, revealing no potential interferences in polyphenols chemical transformations.

In the experiment there were no detections of microbial growth (mesophilic bacteria or yeast) in strawberry purées, confirming that sterile conditions were assured, and consequently the microbial metabolism was not interfering with degradation reactions.

4. Conclusions

Modified atmosphere allowed higher stability on total antioxidant activity, total phenolic and total anthocyanins content of strawberry purée. Among individual phenolic compounds the most affected by atmospheric conditions were anthocyanins, while catechins and quercetin-rutinoside were the most stable. Anthocyanins decreased at a faster rate for samples stored in air.

The conditions that can be recommended for preservation of nutritional properties during purée strawberry storage are refrigerated temperature conditions (4 °C) and an anaerobic atmosphere (100% N_2) since under these conditions the concentration of strawberry colouring polyphenols (anthocyanins) would be higher and compounds like (+)-catechin, (−)-epicatechin, quercetin-3-rutinoside and ellagic acid would be more stable.

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