The effect of thermal processing and storage on the color
stability of strawberry puree originating from different
cultivars

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Highlights

- 12 strawberry cultivars were evaluated for their color and chemical composition stability upon pasteurization and storage
- Thermal processing resulted in anthocyanin degradation, PPO and POD inactivation
- Accelerated storage caused complete vitamin C degradation and loss of red color
- The impact of storage on strawberry puree quality indices was strongly cultivar dependent

Key Words

Strawberry cultivar

Color stability

Processing

Storage

Polyphenol oxidase

Abstract

The effect of cultivar on color (CIELab values, anthocyanin content and % polymeric color), chemical composition (vitamin C, total phenolic content) and enzyme activities during thermal processing and subsequent storage (35 °C for 14 days) as well as their suitability for puree processing was evaluated for twelve strawberry cultivars. Degradation of anthocyanins, color (based on a* and C* values) and total phenols during thermal processing (95 °C, 1 min) was cultivar dependent whereas vitamin C decreased similarly in all cultivars. Despite different levels of POD and PPO activity observed in fresh purees of the different cultivars, these enzymes were completely inactivated in all cultivars after pasteurization. Anthocyanin degradation, which was accompanied by a fade in red color, and loss of vitamin C were remarkable during a 14-day storage period, but the rate differed among the cultivars. In

contrast, polymeric color formation was unaffected by the cultivar. Despite the differences in initial chemical composition, Rendezvous, Senga Sengana and 6082 were the cultivars exhibiting the highest anthocyanin stabilities and lowest % polymeric color values after storage, being good candidates for processing, and suggesting that some intrinsic factors (e.g. copigments) in these cultivars could affect anthocyanin stability.

1 Introduction

Strawberries are fruits rich in health-promoting compounds such as phenolic compounds, vitamin C and folates. In addition to their nutritional attributes, these fruits exhibit highly desirable sensory properties such as unique taste and appealing red color. These sensory and nutritional properties differ strongly between cultivars (Crespo, Bordonaba, Terry & Carlen, 2010; Ngo, Wrolstad & Zhao, 2007). While most strawberries are mainly consumed fresh, around 20% of the total amount harvested is processed into shelf-stable products such as puree, concentrates or juices. Among these, strawberry puree is a key ingredient for many other food products as it provides a tasty flavor and red color when added into smoothies, nectars or fruit preparations.

Color of strawberry-based products is determined by the concentration and type of anthocyanins. The anthocyanin profile can differ with the genotype but pelargonidin-3-glucoside (Pg-3-Glu) followed by cyanidin-3-glucoside (Cyn-3-Glu) and pelargonidin-3-rutinoside (Pg-3-Rut) are reported to be the most abundant anthocyanins in strawberries (Aaby, Mazur, Nes & Skrede, 2012). Aside from the genotype, processing methods and storage conditions influence the anthocyanin stability and hence, the color of the final product. Thermal treatments result in considerable losses of anthocyanins in strawberry puree (Holzwarth, Korhummel, Kammerer, & Carle, 2012). In addition, during ambient and accelerated storage, the red color is rapidly evolving to a dull-brown color (Buvé et al., 2018). Color degradation during storage of shelf-stable strawberry products comprises

71 non-enzymatic reactions such as (i) anthocyanin degradation and polymerization reactions
72 with other phenols (Oliveira, Ferreira, De Freitas, & Silva, 2011) (ii) ascorbic acid (AA)
73 degradation (Es-Safi, Cheynier & Moutounet, 2000) (iii) Maillard associated reactions
74 (Bharate & Bharate, 2014) and (iv) acid catalyzed sugar degradation (Krifi & Maurice, 2000).
75 However, it has been reported by some authors that low residual enzyme activities, mainly
76 attributed to polyphenol oxidase (PPO), could also be responsible for browning of
77 pasteurized strawberry-based products during long-term storage (Gössinger et al., 2009;
78 Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). The PPO activity and thermostability
79 are strongly influenced by the genotype (Chisari, Barbagallo, & Spagna, 2007; Terefe et al.,
80 2013).

81 Few studies evaluated the influence of cultivar on the color stability of strawberry-based
82 products during storage. Strawberry cultivars that were processed into puree or jam following
83 the same conditions exhibited different color stabilities during shelf-life (Mazur et al., 2014,
84 Diamanti et al., 2015). However, detailed information about the correlation between the
85 chemical composition of different cultivars and color stability of the respective
86 strawberry-based product during the manufacturing process and storage is missing in the
87 literature. Diamanti et al. (2015) analyzed 10 different cultivars and suggested that the color
88 was better retained during shelf-life in those cultivars with higher initial anthocyanin and
89 antioxidant contents. However, in the study of Diamanti et al. (2015), neither enzyme
90 activities (PPO) nor non-enzymatic precursors (e.g. AA) were measured. Hence, there is still
91 a need to identify cultivar dependent attributes which are correlated to color stability during
92 processing and storage. Additionally, finding cultivars suitable for industrial processing
93 (i.e. with high color and stability) which can replace the current Senga Sengana cultivar
94 would be beneficial from a sustainability point of view as it is a cultivar that provides a low
95 yield.

To the best of our knowledge, this is the first time a large integrated process-chain experiment involving twelve strawberry cultivars differing in color has been performed aiming at (i) investigating cultivar dependent factors (i.e. chemical composition) related to color stability, (ii) evaluating the impact of processing and storage on the chemical composition and color in these twelve cultivars, and (iii) identifying those cultivars with high color stability suitable for industrial puree processing. For this purpose, the color (CIELab values, anthocyanin profile, % polymeric color), chemical composition (vitamin C, total phenolic content, TPC) and endogenous enzyme activities (PPO and peroxidase (POD)) have been quantified during processing and subsequent accelerated storage (14 d at 35 °C in the dark).

2 Materials & Methods

2.1 Preparation and processing of strawberry purees

Red-fleshed fresh strawberries (*Fragaria x ananassa* Duch.) of the cultivars Clery, Diamante, Honeoye, Lola, Renaissance, Rendezvous, Senga Sengana, Sonata, USA5, 140564 and 6082 were provided by Hansabred GmbH (Dresden, Germany). Additionally, a white-fleshed strawberry cultivar (Snow White) was chosen to gain insights into browning in a different food matrix i.e. a strawberry puree without the presence of anthocyanins. All cultivars were grown on the same test field and harvested during a 3 to 5-wk period (May-June 2019) to achieve the same degree of commercial maturity. To avoid variability between the harvest days the different batches of a single cultivar were mixed into a homogeneous batch and this batch was frozen at -20 °C until processing.

Frozen strawberries were thawed at 4 °C overnight. Calyx and leaves were removed, and fruits were mashed in a Thermomix for 20 s at 10.200 rpm (Vorwerk & Co. KG, Wuppertal, Germany). Seeds were removed using a 500 µm stainless steel sieve at atmospheric conditions. Purees (3.5 kg) were thermally processed in a pilot scale HT220 tubular Heat

exchanger (OMVE, Utrecht, Netherlands). First, the puree was pre-heated to 50 °C and the temperature was further increased to 95 °C. The overall heating up step took approximately 1.5 min. The puree was kept for 60 s at this temperature and afterwards cooled down to 60 °C in 30 s and subsequently to 20 °C in 60 s. Pasteurized purees (165 g) were manually filled, under aseptic conditions, into small polypropylene containers (180 mL) with some headspace in the bottle.

2.2. Storage of purees

Based on Buvé's et al. (2018) study, we chose to use accelerated conditions (35 °C) to study the color stability of strawberry purees. Pasteurized purees were stored at 35 °C in the dark for 14 d with two sampling times after 4 and 14 d. Samples of freshly prepared and pasteurized (0 d) purees were also collected, frozen and kept at -20 °C until analysis.

2.3 Determination of color-related attributes

2.3.1. CIELab values

CIELab values of strawberry purees were measured in reflectance mode with a ColorQuest XE spectrophotometer (Hunterlab, Murnau, Germany), equipped with a D₆₅ illumination source and an 8° observer angle. Measurements were performed in triplicate immediately after sample collection. L^* describes the lightness of the sample, a^* values the greenness/redness and b^* values the blueness/yellowness. Chroma (C^*) and hue angle (h°) were calculated according to Eq. 1 and Eq. 2, respectively. Eq. 2 is only valid for positive a^* and b^* values.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^\circ = \arctan (b^*/a^*) \quad (2)$$

2.3.2 Polymeric color measurements

Polymeric color (PC) was measured in triplicate following the method described by Giusti & Wrolstad (2001). Strawberry puree supernatant (2.8 mL) was diluted with ultrapure water and

then mixed with 0.2 mL of potassium bisulfite solution (0.9 mol/L). The absorbance of the mixture was recorded after 15 min at 420 nm, 496 nm and 700 nm (Evolution 201, Fisher Scientific). The PC was calculated following Eq 3. For color density (K) determination (Eq. 4), the same procedure was followed replacing the bisulfite solution with 0.2 mL of ultrapure water. The PC% was calculated according to Eq. (5).

$$(PC) = [(A_{420nm} - A_{700nm}) + (A_{496nm} - A_{700nm})] \quad (3)$$

$$(K) = [(A_{420nm} - A_{700nm}) + (A_{496nm} - A_{700nm})] \quad (4)$$

$$(PC\%) = \frac{PC}{K} \times 100 \quad (5)$$

2.3.3 Determination of the total and individual anthocyanin content

Anthocyanins were extracted in triplicate using the same extraction procedure as Teribia et al. (2021). Extracts were analyzed using a Dionex BioLC coupled to AD25 UV-Vis detector (Dionex, Sunnyvale, CA, USA). Separation of anthocyanins was performed in a PLRP-S column (250x4.6 mm, 5 μ m and 100 μ m pore size) (Agilent Technologies, Santa Clara, CA, USA), attached to a PLRP-S guard column following the analytical conditions described by Teribia et al. (2021). The flow rate was 1 mL/min and the injection volume 25 μ L. Elution solutions consisted of (A) 4 mL phosphoric acid in 100 mL water and (B) 60 mL acetonitrile in 100 mL water. Anthocyanins were eluted using the following gradient: 0-10 min (10% B); 10-15 min (increase to 18% B); 15-40 min (increase to 25% B); 40-45 min (increase to 100% B); 45-51 min (100% B); 51-52 min (decrease to 10% B); 52-62 min (10% B) and were monitored at 520 nm. Anthocyanins were quantified using an external calibration curve based on Pg-3-Glu standard (Polyphenols, Sandnes, Norway). Assuming a similar response factor, individual anthocyanins were expressed as Pg-3-Glu equivalents.

To identify the individual anthocyanins the mass spectrum of each anthocyanin was obtained using a HPLC system (1200 Series; Agilent Technologies, Diegem Belgium) coupled to a diode array detector and a time-of-flight mass spectrophotometer (6200 Series; Agilent Technologies, Diegem; Belgium) which operated using electrospray ionization. The same column used as described above was operating at 25 °C with a flow rate of 0.5 mL/min. Detection was performed at 520 nm. Formic acid in water 0.1 mL/100 mL (A) and formic acid in acetonitrile 0.1 mL/100 mL (B) were used as eluents and a similar gradient program as before was applied: 0-8 min (5% B); 8-15 min (increase to 10% B); 15-25 min (increase to 15% B); 25-40 min (increase to 20% B); 40-45 min (increase to 60% B); 45-48 min (60% B) 48-49 min (decrease to 5% B); 49-59 min (5% B). Mass spectra were acquired using the same conditions as described by Carrillo, Buvé, Panozzo, Grauwet & Hendrickx (2017). All detected anthocyanins were tentatively identified comparing the masses of the detected anthocyanins with existing literature data (Aaby et al., 2012). The identity of Pg-3-Glu and Cyn-3-Glu was confirmed by comparing the retention time with available commercial standards (Polyphenols, Sadness, Norway).

2.4 Determination of the total vitamin C content

The total vitamin C content was determined with the procedure of Buvé et al. (2018). For the extraction, the same procedure described by Buvé et al. (2018) was followed. The Dionex BioLC equipment used for analyzing and quantifying AA was the same as described in Section 2.3.3. The column used in the analysis was a Prevail C18 column (250x4.6 mm i.d. 5 µm, Grace, Columbia, MD, USA) attached to the corresponding guard column. The mobile phase consisted of 1 mmol/L Na₂EDTA and 10 mmol/L CH₃COONH₄ in water (pH 3.0). The injection volume was 25 µL and the column temperature 25° C. AA was detected at 245 nm and quantified using a calibration curve made of a solution of AA (99% Acros Organics, Geel, Belgium) in the same extraction buffer.

2.5 Determination of total (free) phenolic content

The TPC content of the purees was quantified in triplicate for each sample using the methods described by Singleton & Rossi (1965) and Aaby et al. (2005) with some modifications. Briefly, strawberry puree (5 gr) was homogenized with an extraction solution (5 mL) of aqueous methanol (95 mL/100 mL) with 0.1 mL/100 mL of HCl. The homogenate was centrifuged (8000 g, 15 min at 4 °C), the supernatant was collected and the preceding extraction was repeated for the residue. The pooled supernatants were diluted with water (1:20) and were mixed with 1 mL of previously diluted Folin-Ciocalteu reagent (1:10 v/v) and 0.8 mL of Na₂CO₃ solution. The mixture was incubated in the darkness for 2 h at room temperature. Absorbance was recorded at 765 nm and the total phenols were quantified using an external calibration curve with gallic acid and were expressed as gallic acid equivalents (GAE).

2.6 Determination of POD and PPO activity

2.6.1 POD and PPO extraction

The extraction and partial purification of strawberry PPO and POD was performed following the method described by Teribia et al. (2021).

2.6.2 PPO enzyme assay

The PPO activity was determined using 50 mmol/L catechol in phosphate buffer (0.1 mol/L, pH 5.8) as substrate. The enzyme extract (0.1 mL) was mixed in a cuvette with 1 mL of substrate and the absorbance was immediately recorded at 420 nm in a kinetic mode at 25 °C. The measurements were performed in duplicate. The activity of the enzyme was expressed as the change in absorbance/min/g fresh weight (FW).

2.6.3 POD enzyme assay

The POD activity measurement was adapted from the method described by Terefe et al. (2010) using guaiacol as a substrate. The enzyme extract (0.2 mL) was mixed with 1.5 mL of phosphate buffer (0.1 mol/L, pH 6.5), 0.2 mL of 1 mL/100 mL guaiacol in phosphate buffer (0.1 mol/L, pH 6.5) and 0.2 mL of 1.5 mL/100 mL hydrogen peroxide solution. The absorbance at 470 nm was measured every 8 s for 10 min 25 °C. The activity of the enzyme was expressed as the change in absorbance/min/g FW.

2.7 Statistical analysis

To study the effect of the cultivar, thermal processing and storage as well as the interactions between these factors, a standard least squares regression analysis was performed. The models fitted allowed for a nonlinear relationship between the various attributes under study and the storage, for each cultivar and thermal processing option. For the attributes TAC, total vitamin C and TPC, a desirable fit was obtained assuming a logarithmic relationship with the length of the storage. For the remaining quality attributes, better fits were obtained using a quadratic model with storage time. Model effects were considered significant when their p-values were smaller than 0.05. JMP Pro 15.1 software (SAS Institute, Cary, NC, USA) was used to perform the least squares regression analysis. To study the differences in PPO and POD activity between the cultivars, one-way analysis of variance was performed, followed by a Tukey's post-hoc for pairwise comparisons (Minitab 17.0, Minitab LLC, USA).

3 Results & Discussion

3.1 Stability of color-related attributes during processing and storage

3.1.1 CIELab values

Fresh purees prepared from red-fleshed strawberries (i.e. all cultivars except Snow White) exhibited a^* values ranging from 21 to 30 indicating a high diversity in the initial red color (Table 1). As expected, Snow White showed very low a^* values due to the low amounts of red pigments. In general, the a^* , C^* and h° values of strawberry purees increased after thermal processing. According to the least squares regression analysis, the increase in C^* , a^* and h° values upon thermal processing was significant and cultivar dependent (Table 2), and generally accompanied by an increase in lightness (Table 1).

Storage resulted in a significant decline in a^* and C^* values (Table 1), regardless of the cultivar. Results were in accordance with García-Viguera et al. (1999) reporting reductions of a^* values in strawberry jams produced from different cultivars upon storage for 96 d at 37 °C. The opposite trend was observed for the h° values which significantly increased in all cultivars, except for 6082. Lola, 140564 and Renaissance were the cultivars exhibiting the highest h° values. The increase in h° values and decline of a^* and C^* values during storage were statistically significant and indicated a color change from red to more yellowish tones, attributed presumably to the formation of brown pigments (Holzwarth et al., 2012). Lightness (L^*) did not show a clear increase during storage, which is in agreement with Garzón & Wrolstad (2002). Based on the regression models (Table 2), color changes occurring during shelf-life of strawberry puree were significantly different between the cultivars suggesting that brown color development is strongly influenced by the cultivar. The cultivars 6082, Diamante, Rendezvous, Senga Sengana and USA5 could represent potential candidates for puree processing as they exhibited lower h° values (below 30) at the end of storage and the

color difference (ΔE^*), Δa^* and Δh° values were generally lower compared to the remaining cultivars (Supplementary Material, Table A1). Snow White exhibited particular CIELab values compared to the other cultivars due to the absence of red pigments. However, a clear increase of h° and decline in L^* values was observed throughout the storage indicating a less bright and more dark puree confirming that this puree turned brown as well during accelerated storage.

3.1.2 Total anthocyanin content (TAC)

The TAC differed greatly and significantly across the freshly prepared purees ranging from 30 to 100 mg/100 g FW for 140564 and 6082 cultivars, respectively (**Fig 1A; Table 2**). Other studies have reported similar anthocyanin concentrations in this range for other cultivars (Nowicka et al., 2019; Terefe et al., 2013). Differences between the fresh samples of the cultivars were not only observed for the total content of the pigments, but also for the anthocyanin profile (**Table 3**). Over all cultivars, five anthocyanins were present namely Cyn-3-Glu, Pg-3-Glu, Pg-3-Rut, cynidin-3-malonyl-glucoside (Cyn-3-malonyl-Glu) and pelargonidin-3-malonyl-glucoside (Pg-3-malonyl-Glu). Pg-3-Glu was the most abundant anthocyanin accounting for 70% of the total anthocyanin content on average. These results are in agreement with previous reports (Buendía et al., 2010; Nowicka et al., 2019). Pg-3-malonyl-Glu was also present in high amounts (15% of the TAC) in all cultivars except for Renaissance. These results are consistent with Aaby and co-workers (2012) who studied the anthocyanin profile of 27 cultivars and observed a contribution of 0-33% for Pg-3-malonyl-Glu. Anthocyanins with a diglycosidic moiety such as Pg-3-Rut were present in lower amounts as previously reported (Tulipani et al., 2008).

Pasteurization caused a significant TAC loss between 2 to 14%. The cultivars Renaissance, Rendezvous, Senga Sengana and Sonata showed the highest stabilities (i.e. less than 6% loss) upon thermal processing (**Fig 1A**). This in line with Holzwarth et al. (2012) reporting a 10%

anthocyanin loss after processing in strawberry puree for 3 min at 90 °C and Hartmann, Patz, Andlauer, Dietrich & Ludwig (2008) observing 14% loss of anthocyanins after 2 min at 85 °C. The mechanism for anthocyanin degradation upon thermal treatment has been reviewed in the literature (Patras, Brunton, O'Donnell, & Tiwari, 2010) but it is still not fully understood.

Storage negatively affected the stability of anthocyanins (**Fig 1A**). On average, around 62% of the total anthocyanins were degraded after 4 d of storage at 35 °C, while 89% of them were degraded after 14 d. These results are in agreement with the colorimetric measurement values (section 3.1.1) in which we also observed a sharp decrease during storage. Anthocyanins are highly unstable compounds that degrade during storage and the degradation mechanism, even though it is not fully understood, is presumably attributed to non-enzymatic reactions such as polymerization or condensation reactions with other phenolics (e.g. tannins) and AA (Monagas, Bartolomé, & Gómez-Cordovés, 2005; Oliveira et al., 2011; Stebbins et al., 2016). The regression model suggests that the effect of storage was significant and that the decrease in TAC was cultivar dependent meaning that the anthocyanin stability is strongly influenced by the cultivar (**Table 2**). The cultivars exhibiting the highest storage stabilities were Rendezvous and Senga Sengana both retaining 17% of the total anthocyanins after 14 d at 35 °C and the cultivar 6082 retaining 15% of the total anthocyanins. Senga Sengana has been previously described to exhibit a higher color stability in strawberry jam compared to Blink and Polka cultivars after 6 months of storage at 4 °C and 20 °C (Mazur et al. 2014). Increasing the initial anthocyanin content of strawberry concentrate and juice (i.e. fortification with different anthocyanins) enhanced the stability of the pigments after 6 d of storage at 25 °C probably due to a protective effect of anthocyanins (i.e. self-association reactions between anthocyanins) (Garzón & Wrolstad, 2002). Hence, it could be hypothesized that cultivars with a high initial anthocyanin content would exhibit higher color

stabilities upon storage. This hypothesis was also formulated by Diamanti et al. (2015) who stated that the cultivars with an initial higher anthocyanin content (and antioxidant content) were the most stable after 180 d at refrigerated and ambient temperatures. However, in our study, despite having an initial high anthocyanin content compared to other cultivars, Honeoye was the cultivar which was affected the most by the storage, retaining only 7% of the total anthocyanins after 14 d. The same was true for the cultivars Diamante and USA5 which showed only a retention of 10% despite the high initial anthocyanin content. It could be that either the anthocyanin concentration was not high enough to act as intermolecular stabilizers in self-association reactions, or other factors such as the absence of particular co-pigments in the former cultivars could be contributing to the instability of these pigments during storage (Carle & Schweiggert, 2016). However, when looking into the data in terms of absolute amounts of anthocyanins, the cultivars 6082, Senga Sengana, Diamante and USA5 exhibited the highest TAC values (between 9 to 14 mg/ 100 g FW) after 14 d of storage suggesting that, except for Senga Sengana, those cultivars with a high initial TAC will exhibit a higher anthocyanin content after accelerated storage.

With regard to the stability of the individual anthocyanins, generally, upon thermal processing Cyn-3-Glu and Pg-3-Rut were the most stable anthocyanins (**Table 3**). However, in the first days of storage (0-4 d) Pg-3-Rut exhibited the highest stabilities whereas after 14 d all individual anthocyanins were similarly affected. The structure of the anthocyanin influences the stability of the anthocyanin in a food matrix (Patras et al., 2010). The presence of additional hydroxyl groups on the aromatic B ring is reported to reduce the stability (Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006). In the same manner, the presence of diglycosides (e.g. rutinose) enhances the stability compared to monoglycosides (glycosides) (Sui, Dong, & Zhou 2014). In our study, this was true for the case of Pg-3-Rut. However, Cyn with additional hydroxyl groups compared to Pg, was more stable when the same sugar

moiety was present. Aside from the structure, other factors related to the food matrix such as pH, the presence of AA, metal ions and phenolics can either have a de-stabilizing effect as is the case of AA or enhance anthocyanin stability (e.g. co-pigmentation with other phenolic acids) (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Hence, it can be hypothesized that Senga Sengana and Rendezvous cultivars may potentially have a different phenolic profile conferring stability to anthocyanins which could explain the higher anthocyanin stability for these cultivars after storage. Overall, these results emphasize the importance of choosing the right cultivar for industrial processing exhibiting a high anthocyanin content and red color as well as a high stability throughout thermal processing and accelerated storage. In this context and in terms of stability (% loss) Rendezvous, Senga Sengana and the cultivar 6082 represent good candidates for this purpose. However, when looking into the final anthocyanin content after storage, cultivars such as 6082, Senga Sengana, Diamante and USA5 would be more suitable.

3.1.3 Polymeric color (%)

The PC% is an indicator to which extent polymeric anthocyanins are formed through condensation or polymerization reactions with flavan-3-ols and/or proanthocyanidins (Wrolstad, Durst & Lee, 2005). Initial PC% values were in a wide range, ranging from 8% to 25% in freshly prepared purees for the cultivars Senga Sengana and 140564, respectively (**Fig 1B**). Except for Senga Sengana, Renaissance, USA5 and 6082, the PC% was found to represent more than 10% of the total color in the fresh sample. The cultivars exhibiting PC% values above 10% in the fresh samples could contain dimeric anthocyanins that can exist naturally in strawberries (Fossen et al., 2004).

The values of polymeric color after thermal processing significantly increased (**Fig 1B**). However, this increase varied significantly according to the cultivar (**Table 2**) achieving values above 30% for Honeoye, Sonata, Clery, Lola and 140564. These observations agree

with Ngo et al. (2007) who reported an increase in PC% from 7.2% in frozen fruits to 33% and 27.7% after preparing canned strawberries and jam, respectively.

Storage led to further significant increase of PC% in all cultivars (**Fig 1B; Table 2**). Purees prepared from Senga Sengana, Rendezvous and 6082 showed the lowest PC% values (<60%) at the end of storage. PC% and TAC are often negatively correlated during storage; a decrease in monomeric anthocyanins is accompanied with an increase in PC% (see Section 3.1.2). The regression model suggests that anthocyanin degradation during storage was cultivar dependent, contrary to PC% (**Table 2**). Thus, it can be hypothesized that other reactions aside from polymerization reactions could be involved in anthocyanin degradation as well. AA for example can accelerate anthocyanin degradation directly through condensation reactions (in position C4) or indirectly through the products formed from AA degradation (e.g. H₂O₂, furfural) (Es-Safi et al., 2000; Stebbins et al., 2016).

3.2 Stability of vitamin C during processing and storage

In this study the total vitamin C content was expressed as the sum of AA and dehydroascorbic acid (DHAA) and ranged from 23 mg/100 g FW in the fresh purees prepared from the cultivar 6082 to 41 mg/100 g FW in Honeoye (**Fig 1C**). Generally, pasteurization resulted in small, but significant vitamin C losses (i.e. below 10%), except for Rendezvous (30% loss) (**Table 2**). These observations agree with Holzwarth et al. (2012) who reported limited losses of vitamin C after heating strawberry puree at 90 °C and 75 °C for 3 min. The decline of vitamin C upon thermal processing was similar (i.e. interaction effect of cultivar and pasteurization not significant) across the cultivars (**Table 2**).

The decrease of vitamin C during storage was remarkable in all the cultivars, declining up to 76% on average during the first 4 d of storage (**Fig 1C**). Except for Lola and Clery, vitamin C was completely degraded after 14 d of storage. The low stability of this compound in strawberry-based products upon storage is well-documented (Buvé et al., 2018). The

degradation mechanism is known to involve both aerobic and anaerobic pathways depending on the available oxygen concentration. The products of these reactions such as 2,3-diketogulonic acid, furfural and reductones will further react leading to the formation of brown compounds or can also lead to anthocyanin degradation (Es-Safi et al., 2000). In the present study, the degradation pathway of vitamin C during storage is assumed to be mainly aerobic due to the presence of oxygen during filling and the use of oxygen permeable polypropylene as packing material. As with the TAC, the vitamin C degradation during storage was significantly different across the different cultivars (**Table 2**).

3.3 Stability of TPC during processing and storage

The average TPC for the different freshly prepared purees was 170 mg GAE/100 g FW with Snow White, USA5 and Diamante having the highest amounts (**Fig 1D**). The values for TPC obtained in this study are in agreement with other reports in which different strawberry cultivars were studied (Aaby et al., 2012; Diamanti et al., 2015). It has been reported that white strawberries such as the wild species Chilean strawberry (*Fragaria chiloensis* ssp. *chiloensis*) can be rich in phenolic compounds such as flavonoid glycosides, tannins and ellagic acid derivatives (Simirgiotis et al., 2009). Thermal processing resulted in 5% TPC loss on average. The TPC losses were cultivar dependent (**Table 2**). Snow White and Sonata were the cultivars with the highest losses (12% and 11%, respectively), whereas the cultivars 140564 and 6082 retained 98% and 97% of TPC, respectively.

Storage had notable effects on TPC causing 20% and 33% loss on average for 4 and 14 d of storage, respectively. However, the drop in TPC did not differ significantly across the twelve cultivars (**Table 2**). Strawberry anthocyanins contribute for around 41% of the TPC as reported by Aaby and co-workers (2012), except for Snow White. Hence, the observed decline for TPC during shelf-life is mostly attributed to the decrease on anthocyanins. For the particular case of Snow White, the decrease in TPC could be presumably attributed to the

degradation of flavonoid glycosides and tannins. Degradation of phenolic compounds during storage in strawberry purees has also been reported in literature (Terefe et al., 2013; Holzwarth et al., 2012). The degradation of phenolic compounds could involve several pathways, the most important ones being the oxidation of phenols (e.g. autooxidation by metal ions or light) and the polymerization reactions between phenols (Oliveira et al., 2011).

3.4 PPO and POD activity during processing and storage

The variability in enzyme activity between cultivars in fresh samples was more remarkable for POD compared to PPO (**Fig 2**), but for both cases the differences across the cultivars were statistically significant ($P<0.05$). The highest PPO activity was observed for Renaissance whereas Snow White showed the lowest. Furthermore, in the cultivars 6082 and Snow White no POD activities were surprisingly detected (or below the limit of detection) in contrast to Honeoye which exhibited the highest POD activity (**Fig 2**). The activity and expression of both oxidases is known to be dependent on the ripening stage. Unripe fruits exhibit higher activities compared to fully-ripe fruits (Serradell et al., 2000). Hence, as in present study all cultivars were harvested at optimum commercial maturity stage, we hypothesize that the expression of POD in the cultivars mentioned above in an optimum maturity stage is very low (below the detection limit of the assay).

Pasteurization resulted in an effective PPO inactivation irrespective of the cultivar (results not shown). This result is consistent with the findings of Marszałek, Mitek, & Skąpska (2015) and Teribí et al. (2021) who reported a complete inactivation of PPO after heating strawberry purees for 2 min at 70 °C and for 1 min at 95 °C, respectively. However, some authors reported high thermostability of strawberry PPO which could result in residual enzyme activities after the thermal processing responsible for browning during storage (Terefe et al., 2010; Holzwarth et al. 2012). The differences between the former studies and our study might be related to the analytical procedure to measure the activity of this enzyme.

The majority of literature does not purify the enzyme prior to the activity assay and hence, endogenous components present in the extract may interfere with the spectrophotometric assay overestimating the enzyme activity.

Similar to PPO, no POD activity was detected after pasteurization which is in agreement with previous studies (Chisari et al., 2007; Marzalek et al., 2015). The thermostability of both enzymes is known to be cultivar dependent, however, in this study, a heat treatment of 95 °C for 1 min was sufficient to completely inactivate both PPO and POD in all samples (below detection limit). It was also verified experimentally that the enzymes did not reactivate during storage (data not shown).

4 Conclusion

In the present study, the evolution of quality parameters of strawberry purees from twelve cultivars during thermal processing and subsequent storage were evaluated. The influence of the chemical composition of the cultivars on these changes was also studied. Freshly prepared purees from twelve cultivars exhibited different colors (based on CIELab values, TAC and PC%), chemical composition (i.e. vitamin C content, TPC) and enzyme activities. For all cultivars, pasteurization resulted in significant losses of anthocyanins, vitamin C, TPC, an increase in %PC and complete enzyme inactivation. The extent of these changes was cultivar dependent contrary to vitamin C that decreased similarly in all cultivars. During storage, the degradation of anthocyanins, vitamin C and color-related attributes was cultivar dependent. In contrast, PC% and TPC changes throughout the storage occurred in a similar way for all cultivars suggesting that other reactions aside from polyphenol polymerizations are involved in color loss. In addition, both PPO and POD were completely inactivated after pasteurization suggesting that their role on color loss during shelf-life would be limited. Our results highlight the importance of adequate raw material selection for processing as the color stability is strongly influenced by the cultivar, being 6082, Rendezvous and Senga Sengana

the ones exhibiting the highest color and anthocyanin retentions after storage despite of the initial different anthocyanin concentrations. In terms of anthocyanin content, the cultivars 6082, Diamante and USA5 exhibited the highest contents after storage representing promising cultivars next to the commonly used Senga Sengana to produce a processed product like strawberry puree.

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6 Conflict of interest

Authors declare no conflict of interest

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FIGURE CAPTIONS

Figure 1. (A) Total anthocyanin content (TAC), (B) percentage of polymeric color (PC%), (C) total vitamin C content and (D) total phenolic content (TPC) in fresh (■), thermally processed (▤) and stored 4 (□) and 14 days (■) at 35 °C purees made from different strawberry cultivars.

The results represent the mean and standard deviation (n=3) of samples

Figure 2. Polyphenol oxidase (PPO, ■) and peroxidase (POD, □) activity ($\Delta\text{Abs}/\text{min}/\text{g FW}$) in fresh strawberry purees prepared from 12 cultivars.

The results represent the mean and standard deviation (n=2) of samples. Significant differences ($P<0.05$) between cultivars on PPO and POD activity are represented in lower case letters and capital letters, respectively. cv: cultivar

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648 **Figure 1**

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659 **Figure 2**

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Table 1. Color parameters (a^* , C^* , h° and L^* values) in fresh, thermally processed and stored (4, 14 days) purees made from different strawberry cultivars (cv).

Cultivar	a^* value				C^* value				h° value				L^* value			
	Fresh	0d	4d	14d	Fresh	0d	4d	14d	Fresh	0d	4d	14d	Fresh	0d	4d	14d
cv. 140564	29.65 ±0.01	29.67 ±0.04	20.88 ±0.12	10.97 ±0.05	32.65 ±0.03	32.93 ±0.03	24.14 ±0.18	15.48 ±0.12	24.75 ±0.12	25.73 ±0.04	30.12 ±0.18	44.84 ±0.26	39.64 ±0.38	40.74 ±0.15	41.11 ±0.09	37.92 ±0.03
cv. 6082	23.88 ±0.51	28.83 ±0.80	20.50 ±0.71	12.90 ±0.12	25.84 ±0.58	31.79 ±0.99	22.18 ±0.73	13.98 ±0.14	22.45 ±0.20	24.90 ±0.44	21.84 ±0.23	22.74 ±0.19	31.66 ±0.32	34.13 ±0.51	32.56 ±0.22	31.15 ±0.79
Clery	27.42 ±0.38	29.68 ±0.78	22.05 ±0.10	11.73 ±0.09	26.28 ±0.08	32.83 ±0.93	24.18 ±0.11	14.75 ±0.14	24.19 ±0.30	25.26 ±0.25	24.24 ±0.04	37.36 ±0.19	34.68 ±0.34	37.46 ±0.57	37.27 ±0.12	35.86 ±0.15
Diamante	23.89 ±0.13	34.70 ±0.05	22.02 ±0.08	12.42 ±0.05	25.75 ±0.14	26.62 ±0.03	23.75 ±0.11	14.17 ±0.08	21.89 ±0.10	29.01 ±0.03	22.02 ±0.11	28.84 ±0.18	32.56 ±0.14	34.70 ±0.05	34.42 ±0.06	32.92 ±0.36
Honeoye	21.92 ±0.44	29.22 ±0.33	23.81 ±0.06	12.16 ±0.04	33.94 ±1.35	32.14 ±0.40	26.36 ±0.08	16.22 ±0.08	21.47 ±0.15	24.60 ±0.12	25.37 ±0.06	41.43 ±0.08	34.11 ±0.04	37.35 ±0.22	37.68 ±0.05	36.58 ±0.06
Lola	30.31 ±0.20	33.37 ±0.23	21.00 ±0.06	13.26 ±0.12	33.30 ±0.23	34.48 ±0.03	26.93 ±0.13	18.74 ±0.03	24.70 ±0.17	25.93 ±0.14	30.25 ±0.03	50.24 ±0.06	38.48 ±0.12	42.31 ±0.13	44.20 ±0.07	42.83 ±0.04
Renaissance	30.58 ±1.08	30.97 ±0.06	23.53 ±0.12	11.85 ±0.04	30.06 ±0.48	34.69 ±0.08	26.21 ±0.15	15.66 ±0.05	25.70 ±0.54	26.78 ±0.05	26.16 ±0.08	40.82 ±0.18	36.74 ±0.81	40.58 ±0.15	39.57 ±0.32	37.53 ±0.06
Rendezvous	27.41 ±0.16	29.05 ±0.16	21.91 ±0.23	13.98 ±0.20	30.18 ±0.23	32.40 ±0.21	24.05 ±0.29	16.11 ±0.29	24.77 ±0.21	26.28 ±0.14	24.35 ±0.18	29.85 ±0.41	36.86 ±0.56	40.23 ±0.39	36.46 ±0.26	34.98 ±0.28
Senga Sengana	24.20 ±0.07	26.39 ±0.31	19.75 ±0.04	11.59 ±0.06	26.28 ±0.08	28.86 ±0.36	21.32 ±0.03	13.00 ±0.09	22.91 ±0.10	23.88 ±0.11	22.16 ±0.10	26.90 ±0.19	32.78 ±0.14	34.33 ±0.31	32.73 ±0.13	31.81 ±0.15
Snow White	8.61 ±0.05	7.91 ±0.02	5.09 ±0.09	5.88 ±0.01	15.54 ±0.10	16.89 ±0.02	17.37 ±0.10	16.15 ±0.01	56.35 ±0.24	62.08 ±0.09	72.97 ±0.21	68.65 ±0.03	55.84 ±0.67	60.19 ±0.03	53.80 ±0.51	46.92 ±0.04
Sonata	27.48 ±0.10	28.10 ±0.06	21.57 ±0.08	11.15 ±0.03	30.13 ±0.15	30.90 ±0.06	23.58 ±0.12	13.80 ±0.03	24.24 ±0.19	24.56 ±0.10	23.83 ±0.17	36.08 ±0.28	35.24 ±0.10	36.71 ±0.04	35.65 ±0.09	33.99 ±0.03
USA5	22.83 ±0.20	26.80 ±0.08	21.83 ±0.04	13.09 ±0.04	24.46 ±0.22	29.12 ±0.10	23.33 ±0.03	14.78 ±0.01	21.01 ±0.13	22.99 ±0.06	20.70 ±0.08	27.71 ±0.13	32.04 ±0.12	34.94 ±0.09	33.73 ±0.28	33.33 ±0.04

The results represent the mean and standard deviation (n=3) of samples

Abbreviations: a^* : redness C^* : chroma h° : hue angle L^* : lightness

Table 2. Influence of cultivar, pasteurization and storage time on the concentration and values of selected attributes in strawberry purees based on standard least squares regression models. The effect of the interactions between cultivar and pasteurization (cultivar*pasteurization), and between cultivar and storage (cultivar*storage) are shown as well.

Variables	a* value	C* value	h° value	TAC	PC%	Total Vitamin C	TPC
Cultivar	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Pasteurization	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Storage	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Cultivar*Pasteurization	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	NS	$p<0.001$
Cultivar* Storage	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	NS	$p<0.001$	NS

h°: hue angle TAC: Total anthocyanin content TPC: Total phenolic content PC%: Percentage of polymeric color

NS: not significant

Table 3. Content of individual anthocyanins (mg/100 g FW) in fresh, thermally processed and stored purees made from different strawberry cultivars.

Cultivar	Cyn-3-Glu				Pg-3-Glu				Pg-3-Rut			
	Fresh	0d	4d	14d	Fresh	0d	4d	14d	Fresh	0d	4d	14d
cv. 140564	0.48 ±0.03	0.49±0.02	<LOQ	<LOQ	25.04±0.40	22.76±0.83	6.57±0.55	2.97±0.08	1.11±0.04	1.11±0.06	0.62±0.05	<LOQ
cv. 6082	4.68 ±0.10	4.22±0.05	2.21±0.13	0.74±0.01	58.53±1.19	53.29±0.78	28.81±0.59	11.04±0.28	3.37±0.13	3.03 ±0.08	1.83 ±0.05	0.65 ±0.06
Clery	0.47±0.04	0.46±0.03	<LOQ	nd	38.33±0.27	35.76±0.79	12.00±0.09	3.32±0.05	1.18±0.02	1.29±0.25	0.62±0.01	<LOQ
Diamante	6.31±0.15	5.67±0.05	2.69±0.03	0.65±0.002	57.38±1.44	49.77±0.66	23.21±0.18	7.71±0.22	2.04±0.18	1.71±0.06	0.94±0.01	<LOQ
Honeoye	1.47±0.02	1.41 ±0.02	0.55 ±0.01	<LOQ	48.28±0.74	45.13±0.73	17.25±0.02	4.08±0.08	2.66±0.05	2.44±0.04	1.15±0.07	<LOQ
Lola	0.49±0.02	0.46±0.01	<LOQ	nd	23.43±0.35	21.07±0.70	6.27±0.39	2.33±0.02	1.84±0.22	1.75±0.18	0.79±0.07	<LOQ
Renaissance	2.74±0.04	2.68±0.04	1.05±0.002	<LOQ	41.12±0.68	40.28±0.73	14.68±0.13	4.00±0.08	nd	nd	nd	nd
Rendezvous	1.07±0.03	0.93±0.01	0.47±0.003	<LOQ	29.12±0.87	26.42±0.20	11.91±0.15	5.2±0.09	2.41±0.06	2.05±0.05	1.32±0.08	0.44±0.01
Senga Sengana	4.89±0.29	4.74±0.27	2.7±0.01	nd	58.70±3.14	55.57±3.69	29.81±0.15	11.56±0.21	3.19±0.56	3.06±0.28	2.03±0.02	0.78±0.02
Snow White	0.38±0.01	0.31±0.01	nd	nd	0.28±0.003	<LOQ	nd	nd	nd	nd	nd	nd
Sonata	1.39±0.03	1.38±0.05	0.59±0.01	nd	47.11±0.65	44.08±1.73	17.67±0.39	4.55±0.13	2.36±0.03	2.16±0.08	1.01±0.02	<LOQ
USA5	5.06±0.28	4.75±0.05	2.44±0.04	0.51±0.02	54.90±1.54	50.53±0.56	25.42±0.54	7.33±0.06	nd	nd	nd	nd

Table 3. Continued

Cultivar	Cyn-3-malonyl-Glu				Pg-3-malonyl-Glu			
	Fresh	0d	4d	14d	Fresh	0d	4d	14d
cv. 140564	nd	nd	nd	nd	5.01±0.11	4.54±0.19	1.47±0.01	<LOQ
cv. 6082	2.16 ±0.05	1.87±0.03	0.84±0.06	nd	31.6±0.67	27.22±0.49	11.0±0.26	2.21±0.03
Clery	nd	nd	nd	nd	12.5±0.01	10.76±0.22	3.52±0.03	0.44±0.001
Diamante	2.73±0.07	2.25±0.06	0.91±0.002	nd	26.3±0.59	21.60±0.27	8.24±0.07	1.43 ±0.01
Honeoye	0.39±0.01	0.34±0.001	nd	nd	11.70±0.18	10.47± 0.14	3.71±0.03	0.45±0.03
Lola	nd	nd	nd	nd	6.34±0.16	5.42±0.26	1.71±0.30	0.22±0.001
Renaissance	nd	nd	nd	nd	nd	nd	nd	nd
Rendezvous	0.30±0.001	nd	nd	nd	10.52±0.27	10.04±0.17	3.99±0.02	0.71±0.02
Senga	0.70±0.08	0.65±0.05	nd	nd	10.30±0.53	9.58±0.48	3.78±0.001	0.74±0.01
Sengana	0.49±0.01	0.41±0.004	<LOQ	<LOQ	<LOQ	<LOQ	nd	nd
Snow White								
Sonata	0.32±0.001	0.30±0.01	nd	nd	10.60±0.15	10.42±0.34	3.73±0.06	0.44±0.004
USA5	2.62±0.07	2.25±0.05	0.93±0.01	nd	27.0±0.69	23.24±0.20	9.21±0.17	1.30±0.02

All values are expressed as Pg-3-Glu equivalents. The results are the means and standard deviation (n=3) of samples

Abbreviations: Cyn-3-Glu: Cyanidin-3-glucoside Pg-3-Glu: Pelargonidin-3-glucoside Pg-3-Rut: Pelargonidin-3-rutinoside Cyn-3-malonyl-Glu: Cyanidin-3-malonyl-glucoside Pg-3-malonyl-Glucoside: Pelargonidin-3-malonyl-glucoside d: days of storage nd: not detected <LOQ: below limit of quantification (1.2 µg/mL)