**“Impact of processing and storage conditions on color stability of
strawberry puree: the role of PPO reactions revisited”**

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Abstract

The effect of pre-heating fresh strawberries (hot break) and the use of refrigerated temperatures prior to pasteurization on the stability of anthocyanins, vitamin C, color and polyphenol oxidase (PPO) activity during storage (42 days at 35 °C) of strawberry puree was studied. Hot break resulted in 20% residual PPO activity and caused 10% anthocyanin degradation, whereas vitamin C was unaffected. After mashing, purees were stored at 4 °C and 25 °C for 3 hours. During this period, anthocyanins and PPO activity remained constant independently of the processing history but ascorbic acid was oxidized faster at 25 °C. Pasteurization caused complete inactivation of PPO, reduction of anthocyanins (25%), of a^* value (6%) and of vitamin C (50%). Neither partial inactivation of PPO early in the processing (hot break) nor the use of refrigeration prior to pasteurization had a positive effect on color and anthocyanin stability of strawberry puree during subsequent storage, suggesting that PPO-derived reaction products formed during processing have a very limited impact on color degradation during shelf-life.

Highlights

- Different strawberry puree manufacturing processing conditions were evaluated
- Hot break did not improve color stability during further processing and storage
- Refrigeration prior to pasteurization prevented ascorbic acid oxidation
- PPO reactions before pasteurization do not affect color degradation during storage

Key Words

Strawberry

Color stability

41 Polyphenol oxidase

42 Processing

43 Storage

44 Kinetics

45 **List of abbreviations**

46 AA Ascorbic acid

47 ASLT Accelerated shelf-life test

48 CB_4 °C Cold break treatment incubated at 4 °C

49 Control_25 °C Control treatment incubated at 25 °C

50 DHAA Dehydroascorbic acid

51 FW Fresh weight

52 HB_25 °C Hot break treatment incubated at 25 °C

53 K Color density

54 PC Polymeric color

55 PC% Percentage of polymeric color

56 PPO Polyphenol oxidase

57 TAC Total anthocyanin content

List of symbols

59	a^*	greenness to redness
60	b^*	blueness to yellowness
61	C	response at time t
62	C_{∞}	stable response as a function of time
63	C_0	initial response (before storage)
64	C_{ae}	response in the aerobic phase
65	C_{an}	response in the anaerobic phase
66	C^*	chroma
67	h°	hue angle
68	k	apparent reaction rate constant
69	L^*	lightness
70	k_{ae}	apparent reaction rate constant in aerobic conditions
71	k_{an}	apparent reaction rate constant in anaerobic conditions
72	SEE	Standard error of estimate
73	R^2_{adj}	adjusted coefficient of determination
74	RSD	Relative standard deviation
75	t	storage time (days)

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1 Introduction

Strawberry (*Fragaria x ananassa*) is a fruit consumed worldwide and is very attractive for consumers due its unique flavor, bright red color, and nutritional attributes. Vitamin C, folate and, especially, phenolic compounds such as the red coloured anthocyanins are found in abundance (Giampieri et al., 2012). Strawberries are consumed either fresh or processed into various foods/ingredients such as concentrate, juice and puree thereof. For the food industry, strawberry puree represents an interesting natural ingredient to prepare red-colored products with a tasty flavor such as fruit preparations, ice creams, smoothies etc. Nevertheless, the use of strawberry puree in these products is limited by its rapid color degradation occurring during processing and storage (Gössinger et al., 2009a).

Common industrial processes for the production of strawberry puree consists of different steps (i) fruit washing and sorting, (ii) fruit mashing, (iii) sieving as an optional step (iv) a waiting time in which the puree is stored in buffer tanks for 3-5 hours prior to pasteurization and finally (v) pasteurization to ensure the microbial safety and inactivation of quality degrading enzymes within the puree. Color changes occurring in the manufacturing process of strawberry puree results from a combination of brown color development and fading of red color due to anthocyanin degradation (Skrede et al., 1992). Both non-enzymatic and enzymatic reactions have been described to take part in this complex process (Martinez and Whitaker, 1995; Bharate and Bharate, 2014). Non-enzymatic browning, mainly occurring during storage after pasteurization, involves several reactions such as ascorbic acid (AA) degradation, polyphenol oxidation and polymerization reactions, Maillard associated reactions and acid-catalyzed sugar degradation (Poei-Langston and Wrolstad, 1981; Krifi and Maurice, 2000; Oliveira et al., 2011). Enzymatic reactions are primarily attributed to the activity of polyphenol oxidase (PPO) an oxidoreductase that requires oxygen to catalyze the hydroxylation of monophenols to diphenols, and further on, the oxidation of the resulting

diphenols into o-quinones. The quinones formed rapidly react non-enzymatically, with other quinones, AA, proteins and phenols (including anthocyanins) producing undesirable brown compounds or causing discoloration of anthocyanins (Kader et al., 1998, 1999; Es-Safi et al., 2000; Bittner, 2006). However, this enzymatic reaction only takes place upon cell disruption (i.e. fruit mashing) when the substrates located in the vacuoles, and PPO present in the chloroplast thylakoid membrane, come into contact.

To minimize enzymatic browning and guarantee microbial safety, a thermal treatment such as pasteurization is often used. Complete inactivation of PPO upon thermal treatments in strawberry has been previously described in the literature (Dalmadi et al., 2006; Chisari et al., 2007; Terefe et al., 2013). However, Gössinger et al. (2009a) observed residual PPO activities after a thermal treatment (90 °C, 60 min) and hypothesized these to be the cause of browning in stored strawberry nectar. An approach to inactivate enzymes at the start of the production process (often used in the production of dried or frozen foods) is to perform a thermal treatment prior to processing, i.e. on the intact fruits. This step is known as blanching or hot break (HB). Complete PPO inactivation after blanching fresh fruits has been previously observed by Rossi et al. (2003) and Guiamba and Svanberg (2016) in blueberry juice and mango puree, respectively. Rossi et al. (2003) reported an improved color after processing for a juice made from blanched blueberries. However, few authors have evaluated the effect of blanching of fresh fruits on color stability during the shelf-life of a food product derived thereof. Wrolstad et al. (1980) found that blanching frozen strawberries in a microwave (82 °C, 3-4 min) better preserved the color of strawberry concentrate during 8 weeks of storage at 20 °C. However, in the former study PPO activity and its effects on color changes were not evaluated. Hence, despite of the mentioned reports, the impact of the enzyme-derived products formed prior to pasteurization on color degradation during storage remains unclear and should be further investigated. Aside from heat treatments, the use of

refrigerated temperatures (4 °C) during storage can assist to preserve the color (Gössinger et al., 2009b) probably due to the low rates of enzymatic and oxidation reactions. In a similar way, the use of refrigerated conditions, rather than ambient temperature, during the waiting time prior to pasteurization could potentially help to retain the color of disintegrated fruits by reducing the enzymatic activity. However, no reports are available regarding the effect of refrigerated temperatures at this stage of the processing chain on color stability of strawberry puree.

Despite the numerous studies evaluating the color stability of strawberry products during shelf-life, the rapid color degradation (i.e. browning phenomenon) is still a major issue for the food industry. The evaluation of the impact of industrially relevant steps as the waiting time, the influence of the temperature as well as the role of PPO reactions during processing could provide insights and knowledge to optimize and improve the color stability of strawberry-based products and mitigate brown discoloration. This study aimed at (i) evaluating the impact of each industrial relevant processing steps (i.e. sieving, waiting time and pasteurization) on relevant color-related attributes and vitamin C content, (ii) comparing the use of refrigerated (4 °C) and ambient conditions (25 °C) during the waiting time and its impact on color changes during subsequent processing and storage, and (iii) investigating the contribution of PPO-related reactions occurring throughout the processing chain, to browning during shelf-life of pasteurized strawberry puree. To obtain insights in the contribution of PPO, a pre-heat treatment (HB step) was applied to fresh strawberries to inactivate the enzyme. Furthermore, kinetic modelling was performed to quantitatively describe color changes in terms of CIELab values, total anthocyanin content (TAC), percentage of polymeric color (PC%), and non-enzymatic browning reaction precursors (AA) during storage at 35 °C for 42 days.

2 Materials & Methods

2.1 Preparation and processing of strawberry purees

Fresh strawberries (*Fragaria x ananassa*, cv. Renaissance) were provided by Hansabred GmbH (Dresden, Germany) and harvested in a full-ripe stage, on the same day in June 2019. After removing the sepals, 30 kg of fresh strawberries were packed in vacuum-sealed plastic bags (containing a single layer of fruits). Starting from these strawberries, three processing flows were carried out as summarized in **Fig 1**. These three processes will be referred to as Control_25 °C, Cold Break_4 °C (CB) and Hot Break_25 °C (HB). For the HB treatment, 10 kg of fresh fruits without sepals, packed in vacuum sealed plastic bags, were pre-heated in a water bath (95 °C, 8 min) followed by immediate cooling in ice water. Both the HB-treated and non-HB treated fruits (i.e. strawberries which were not heated) were frozen in liquid nitrogen and stored at -40 °C until further processing.

In a next step, pre-heated (HB) and non-preheated fruits were thawed at 4 °C overnight. The pre-heated strawberries were the starting material for the HB processing flow. Non-heated strawberries were used in the control and CB processes. For each process, strawberries were mashed in a Thermomix (Vorwerk & Co. KG, Wuppertal, Germany) for 20 sec at 10.200 rpm and the resulting puree was passed through a 500 µm stainless steel sieve to remove seeds (under atmospheric conditions). The temperature of the puree during this process did not exceed 4 °C. Strawberry purees were filled in 200 mL glass jars (165 ± 1 g). Non-preheated samples (i.e. non-HB) were incubated for 3 hours (i.e. waiting time) at two different temperatures namely 4 °C and 25 °C and will be referred to as cold break (CB_4 °C) and Control_25 °C, respectively. The 3-hour incubation at 25 °C (i.e. control_25 °C) represents the standard manufacturing processing condition of strawberry puree. Purees made of HB fruits were incubated for 3 hours at 25 °C (HB_25 °C). After incubation, all samples were

pasteurized in a pilot-scale water cascading retort system (Barriquand Steriflow, Paris, France). An industrially-relevant process value of $F_{95\text{ }^{\circ}\text{C}}^{10\text{ }^{\circ}\text{C}} = 1\text{ min}$ was selected. The temperature profile at the coldest point of the puree was monitored using T-thermocouples (Ellab, Hillerød, Denmark), see Supplementary Material **Fig A1** for the temperature profile. Preparation and pasteurization of purees was performed in triplicate for each condition (Control_25 °C, CB_4 °C, HB_25 °C). The variability for the thermal processing, measured as relative standard deviation (RSD), was below 10% for all measured attributes in all samples except for total vitamin C and AA. The higher RSD for vitamin C could be explained by the instability of this compound to high temperatures and presence of oxygen during the thermal processing. To study the impact of each processing step, samples were collected at different points for the three different process flows: before sieving, after sieving (0 hours), after waiting time (3 hours) and after pasteurization (0 days) (**Fig 1**). These samples were immediately frozen with liquid nitrogen and stored at -40 °C until analysis.

2.2 Storage of strawberry purees

Glass jars filled with pasteurized strawberry puree were stored at accelerated conditions (35 °C) in the dark for 42 days. For each treatment, 2 jars were randomly sampled from the incubator at 7 different time moments during storage. Samples were collected at 0, 2, 4, 8, 16, 32 and 42 days of storage. After sampling, samples were frozen with liquid nitrogen and kept at -40 °C until analysis.

2.3 Determination of CIELab values

CIELab values of the strawberry purees were measured with a calibrated Hunterlab ColorFlex EZ colorimeter (Hunterlab, Reston, Virginia, USA), equipped with a D₆₅ illumination source, a 10° observer angle and a 45°/0° geometry. The L^* value describes the lightness, the a^* value the greenness-redness and the b^* value the blueness-yellowness of the

sample. The chroma (C^*) and hue angle (h°) were calculated using Eq. (1) and (2), respectively.

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

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

2.4 Polymeric color measurements

Polymeric color (PC) was determined, with the procedure described by Giusti and Wrolstad (2001), by mixing diluted strawberry puree with 0.2 mL of potassium bisulfite (0.9 M). For color density (K) determination, potassium bisulfite was replaced with 0.2 mL of miliQ water. After 15 min of equilibration, absorbance values were recorded in triplicate at 420 nm, 496 nm ($\lambda_{\max}$) and 700 nm. PC and K were calculated using Eq. (3) and (4), respectively. Finally, the percentage of polymeric color ($PC\%$) which is an indicator of anthocyanin polymerization was calculated according to Eq. (5).

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

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

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

2.5 Determination of the total anthocyanin content

TAC in strawberry puree was extracted following the method described by Buvé et al. (2018). Extracts were stored at -80 °C for further analysis. This extraction procedure was performed in triplicate for each sample.

Separation of anthocyanins was carried out using a Dionex BioLC system coupled to a AD25 UV-Vis detector (Dionex, Sunnyvale, CA, USA) and equipped with a PLRP-S column, of 250x4.6 mm, with a 5 μ m particle size and 100 Å pore size, attached to a PLRP-S guard

column (Agilent Technologies, Santa Clara, CA, USA). The column temperature was kept at 25 °C and a constant flow rate of 1 mL/min was applied. Mobile phases consisted of (A) 4% phosphoric acid and (B) 60% acetonitrile in water. The gradient elution was as follows: 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). The injection volume was 25 µl and the absorbance was measured at 520 nm. Anthocyanins were quantified using an external calibration curve based on pelargonidin 3-glucoside (Polyphenols, Sandnes, Norway), dissolved in the same extraction solution.

2.6 Determination of the total vitamin C content

The total vitamin C content consisting of L-ascorbic acid (AA) and dehydroascorbic acid (DHAA), was determined with the method described by Buvé et al. (2018). Briefly, strawberry puree was extracted in a ratio 1:3 (w/v) with an extraction buffer (pH 2.0) composed of 1% m-phosphoric acid (w/v) and 0.5% oxalic acid (w/v). After centrifugation (15 min, 24000 g, 4 °C), the supernatant was collected, and the pH was adjusted to 3.5 using 1 M NaOH. The extraction was performed in triplicate for each sample. To determine the AA content, part of this supernatant was mixed in a ratio 1:2 with phosphate buffer (20 mM NaH₂PO₄ with 1 mM Na₂EDTA, pH 3.5) and filtered. For the total vitamin C content, part of the pH-adjusted supernatant was homogenized, in a ratio 1:2, with 2.5 mM tris-2-carbonyl-ethyl phosphine in phosphate buffer (pH 3.5) to reduce the DHAA present in the extracts into AA. The mixture was centrifuged for 15 min at 19900 g and 4 °C and was filtered through a 0.45 µm syringe filter. The same Dionex BioLC equipment described in section 2.5 was used to analyze AA following the same conditions and column as described by Buve et al., (2018).

2.7 Determination of polyphenol oxidase (PPO) activity

2.7.1 Enzyme extraction

PPO was extracted according to the method described by Dalmadi et al. (2006) with minor modifications. Strawberry puree was mixed with phosphate buffer (0.1 M, pH 7.0) and 4% PVPP in a ratio of 1:2 (w:v) and was kept in an ice bath for 3 min and centrifuged at 9000 g, 4 °C for 15 min. The resulting supernatant was discarded, and the pellet was washed with miliQ water. After centrifugation (9000 g, 4 °C, 15 min) the pellet was extracted (3 hours, 4 °C) with 0.5% Triton X-100 (v/v) and 1 M NaCl in phosphate buffer (0.1 M, pH 7.0). The homogenate was centrifuged at 13800 g, 4 °C for 30 min and the resulting supernatant was subjected to 30-80% ammonium sulfate precipitation. The precipitate was solubilized in phosphate buffer (0.1 M, pH 7.0) and dialyzed (3.5 kDa cut-off membrane) with the same buffer overnight at 4 °C. After dialysis samples were stored at -80 °C until analysis.

2.7.2 Enzyme activity measurement

PPO activity was determined using catechol as a substrate (Dalmadi et al., 2006). Briefly, 1 mL of 50 mM catechol in phosphate buffer (0.1 M, pH 5.8) was mixed with 0.1 mL of enzyme extract. The absorbance at 420 nm was recorded at 25 °C in a kinetic mode. The enzyme activity of those samples stored at 4 °C for 3 h (CB_4 °C) during processing (i.e. during the waiting step) were measured at 25 °C and 4 °C using a thermostat (Thermo Fischer Scientific, Waltham, USA) to have an idea on actual PPO activity during the waiting period at 4 °C. PPO activity was calculated from the linear part of the curve and expressed as OD_{420nm}/min/g FW. The assay was performed in duplicate. The residual activity was calculated from Eq. (6) being A₀ the enzyme activity in the control_25 °C_0h sample.

$$\text{Residual Activity \%} = \frac{A}{A_0} \times 100 \quad (6)$$

2.8 Kinetic modelling of changes during storage

For each processing flow (control_25 °C, CB_4 °C and HB_25 °C) changes in CIE Lab values, PC%, total anthocyanin content and total vitamin C content occurring during storage in strawberry puree were kinetically modeled respectively by a first order (Eq. 7), first order fractional conversion (Eq. 8) or a first order biphasic model (Eq. 9). To identify the appropriate model, a visual inspection of the model fit was performed, combined with the residual plot and the estimation of coefficients of determination (R^2_{adj}) and standard error of estimate (SEE) (Xanthopoulos et al., 2012). All kinetic parameters were estimated with SAS software using non-linear regression (SAS v9.4, 2013, SAS Institute, Cary, NC, USA).

$$C = C_0 \exp(-kt) \quad (7)$$

$$C = C_\infty + (C_0 - C_\infty)\exp(-kt) \quad (8)$$

$$C = C_{ae} \exp(-k_{ae}t) + C_{an} \exp(-k_{an}t) \quad (9)$$

In these equations, C represents the response of the attribute studied at time t (days), C_0 is the initial response at the beginning of storage, C_∞ is the final response value at extended time and k is the apparent reaction rate constant (days^{-1}). The degradation of vitamin C during storage was modelled using a first order biphasic model (Eq. 9) (Oey et al., 2006) in which C_{ae} corresponds to the concentration that reacts aerobically and k_{ae} and k_{an} (days^{-1}) are the apparent reaction rate constants in aerobic and anaerobic conditions, respectively. The concentration that reacts anaerobically is calculated from $C_0 - C_{ae}$ and is referred to as C_{an} .

2.9 Statistical analysis

The effect of each processing step (sieving, waiting time and pasteurization) and processing condition (HB_25 °C, CB_4 °C, control_25 °C) on the measured properties was statistically evaluated using an two-way ANOVA followed by a *post hoc* Tukey HSD test. The processing conditions and steps were considered as the main factors. In all cases, significant

differences were considered when $P < 0.05$. The statistical analysis was conducted using MiniTab 17.3.1 (Coventry, UK). The graphics were created in SigmaPlot 12.0 (Systat Software, California, USA).

3 Results and Discussion

In this study, sieving did not have an effect on the measured attributes: PPO activity, TAC, CIELab values, PC%, AA and DHAA (data not shown). Hence, in the following section the effects of the waiting time and pasteurization as well as storage, for each processing condition, on the different properties are discussed.

3.1 PPO activity during processing

The PPO activity was determined for the freshly prepared puree (0 h), the puree after the waiting time step (3 h) and after pasteurization for the three different processing conditions (**Fig 2A**). The activity in the freshly prepared non-HB puree (control_25 °C 0 h) was 0.026 ± 0.005 OD_{420nm}/min/g FW. The pre-heat treatment (HB at 95 °C, 8 min) applied on the fresh strawberries resulted in a 20% residual enzyme activity. The temperature recorded with a wireless data logger (Ellab, Hillerød, Denmark) in the core (center) of the fruits reached 85 °C during the 8 minutes which might not be sufficient to completely inactivate the enzyme (Supplementary Material, **Fig A2**). The results are in agreement with a previous study of Holzwarth et al. (2012) who observed 41.5 and 32.4% residual PPO activities after steaming fresh strawberries from Elsanta and Everest cultivars at 100 °C for 3 min. During the waiting time, different PPO activities were observed depending on the incubation temperature. Slightly lower, but significantly different, residual PPO activities were observed for CB_4 °C compared to control_25 °C when the activity assay was performed at 25 °C. However, when the enzymatic assay was performed at 4 °C the residual PPO activity was approximately 5 times lower compared to 25 °C. For this case, the enzyme showed a slightly lower activity

during the waiting time compared to HB_25 °C. This means that keeping the purees at 4 °C during processing might be useful alternative to applying a HB step on fresh fruits in order to minimize PPO activity and associated reactions. Few studies evaluated the effect of refrigerated temperatures on enzymatic activity at this stage of the processing flow for strawberry-based products. Chisari et al. (2007) revealed *in vitro* low residual PPO activities at 4 °C of 32% and 16% for Madame Moutot and Elsanta cultivars, respectively, compared to 25 °C.

As can be seen from **Fig 2A**, pasteurization (process value of $F_{95\text{ }^{\circ}\text{C}}^{10\text{ }^{\circ}\text{C}} = 1 \text{ min}$) caused a complete inactivation of PPO in all samples (below detection limit of the method used). Previous works reported complete thermal inactivation of strawberry PPO after 10 min at 65 °C (Dalmadi et al., 2006) and 2 min at 88 °C (Terefe et al., 2013). The differences between the HB and pasteurization on PPO activity can be explained by the thermal impact of the thermal processing applied (milder in the HB) and/or the lower exposure of the enzyme to the heat in the fruit compared to a disintegrated fruit (i.e. puree) (Sulaiman and Silva, 2013).

3.2 Changes in vitamin C during processing

Vitamin C can be present in food products in two forms as AA (reduced form) and DHAA (oxidized form). The total content of vitamin C in the non-HB freshly prepared puree (control_25 °C 0 h) was about 40 mg/100 g FW with AA and DHAA accounting for 75% and 25% of the total content, respectively (**Fig 2B-D**). The total vitamin C content (**Fig 2B**) was unaffected by the HB step, which is consistent with Guiamba and Svanberg (2016) findings after blanching mango pieces at 90 °C for 4 min. However, our results contradict to Holzwarth et al. (2012) who reported losses of vitamin C after steaming fresh strawberries for 3 min at 100 °C. The differences between both studies may be explained by the differences in

the thermal impact of the thermal treatments (i.e. 100 °C for 3 min compared to 95 °C for 8 min). During the 3 hours of waiting time, the total vitamin C content significantly declined by 8% in the samples stored at 25 °C (i.e. control_25 °C and HB_25 °C) (**Fig 2B**). Similarly, the AA concentration decreased in all samples, but more markedly in the control_25 °C. The opposite tendency was observed for DHAA in which there was an increase of 264%, 249% and 140% in the control_25 °C, HB_25 °C and CB_4 °C, respectively after 3 hours of incubation (**Fig 2D**). These results indicated that during the waiting time, in the presence of oxygen, AA was converted into DHAA and this reaction occurred faster at 25 °C compared to 4 °C for the non-HB samples. The higher content of DHAA present after 3 hours in control_25 °C compared to HB_25 °C could be explained by the higher PPO activity observed in the control_25 °C (**Fig 2A**). AA is one of the most used anti-browning agents in the food industry due to its antioxidant properties. As antioxidant, AA is able to catalyze the reduction of the enzymatically formed quinones to their phenolic precursors (Ali et al., 2015) thereby oxidizing AA to DHAA. In addition, the possible presence of an active ascorbate peroxidase, in the control_25 °C samples, responsible for the oxidation of AA in the presence of H₂O₂, could explain these results (Cruz-Rus et al., 2011). Hence, based on the results of this study to prevent the oxidation of AA during puree production refrigerated temperatures (4 °C) are recommended from crushing to the pasteurization. The implementation of low storage temperatures (i.e. 4 °C) compared to room temperature (20 °C) also slowed down the degradation of AA in strawberry jams (Mazur et al., 2014). Alternatively, a temperature of 25 °C can be allowed for in case a HB step is previously performed.

Pasteurization lead to a decrease of about 50% total vitamin C content (**Fig 2B**). CB_4 °C exhibited slight but significant higher vitamin C levels after thermal processing, probably due to the higher content before pasteurization (CB_4 °C 3 h). Losses of vitamin C upon thermal treatments in strawberry-based products have been reported in the literature (Klopotek et al.,

2005; Hartmann et al., 2008) and have been attributed to the low stability of this compound to high temperatures (Verbeyst et al., 2013), in particular in presence of high initial headspace oxygen concentration (Van Bree et al., 2012).

3.3 Changes in color-related attributes during processing

3.3.1 Total anthocyanin content and Polymeric color (%)

Two anthocyanins were present in the strawberry puree made from Renaissance cultivar; pelargonidin-3-glucoside was the most abundant followed by cyanidin-3-glucoside. The TAC, expressed as the sum of the two individual anthocyanins, was 62 mg/100 g FW in the control_25°C 0 h (**Fig 3A**). Aaby et al. (2012) reported contents of anthocyanins ranging from 8.5 to 66 mg/100 g FW depending on the strawberry cultivar. The HB step caused a 10% loss on anthocyanin content compared to the control_25°C 0 h. However, this decrease was not accompanied by an increase in PC% (**Fig 3B**). Similar losses of anthocyanins were reported by Holzwarth et al. (2012) during steam blanching. Anthocyanin losses during HB could be due to the degradation of the pigments at high temperatures (Patras et al., 2010).

During the waiting time, independently of the temperature and processing condition (HB, CB and control), the TAC remained constant (**Fig 3A**). With regards to PC%, the waiting time resulted in a minor, but significant increase, from 11% to 18% in the control_25 °C, while the PC% did not change significantly for the other two processing conditions.

Pasteurization caused on average a 25% reduction in anthocyanin content for all samples. Similar losses were reported by Hartmann et al. (2008) and Terefe et al. (2013) in strawberry puree. The degradation mechanism of anthocyanins in strawberry-based products upon thermal treatment has been reviewed (Patras et al., 2010). The exact mechanism is still unclear but Sadilova et al. (2007) proposed a pH-dependent mechanism with the opening of pyrylium ring as the first step. The resulting chalcone glycoside is deglycosylated forming a

chalcone which is further degraded into the corresponding phenolic acid and phenolic aldehyde. In agreement with Howard et al. (2014), the PC% did not increase after the thermal processing meaning that anthocyanin loss was caused mainly due to oxidation reactions rather than polymerization reactions. Sablani et al. (2010) found no positive effect of blanching on anthocyanin retention after pasteurization (92 °C, 2 min) of a juice/puree prepared from blanched raspberry fruits. However, the opposite effect was observed when preparing juice/pure from blanched blueberries. Similarly, higher anthocyanin retention in juices made from blanched blueberries compared to unheated fruits were reported by Brownmiller et al. (2008). In the former study, the higher retention in the blanched juice was presumably attributed to a lower PPO activity, that could prevent anthocyanin degradation through oxidative coupling reactions involving quinones or quinone-phenols (Kader et al., 1998, 1999).

3.3.2 CIELab values

Color is an important quality attribute that often determines the consumer acceptance of strawberry based products. Redness (a^*) and chroma (C^*) hardly increased after HB, and hue angle (h°) was unaffected by heating fresh fruits (HB step) (**Fig 3C-D, F**). However, there was a significant increase in L^* from 27 to 33 (**Fig 3E**), indicating that the puree turned brighter, which could be related to the formation of white foam during the heat treatment as observed by Holzwarth et al. (2012). Howard et al. (2014) suggested that an increase of lightness could also be associated to anthocyanin decolorization. Similar to TAC, during the 3 hours waiting time, a^* and C^* remained fairly constant independently of the temperature. However, a small but significant decrease in L^* was observed for the HB_25 °C and CB_4 °C purees during the waiting time. Anthocyanin degradation during pasteurization resulted in fading of the red color as shown by the instrumentally measured a^* values which significantly decreased (around 6%) in all samples. Similar observations were reported by

Patras et al. (2009) in thermally treated strawberry puree. C^* and h° values were generally decreased slightly after pasteurization in all samples (**Fig 3E-F**), independently of the processing condition.

Overall, despite the lower PPO activities in HB samples, the TAC and color remained similar in all samples during and after processing, suggesting that anthocyanin degradation and color changes during processing might not be primarily attributed to PPO catalyzed reactions as suggested previously (Rossi et al., 2003; Brownmiller et al., 2008).

3.4 Changes in vitamin C during storage

After the pasteurization step, strawberry purees were stored at accelerated conditions (42 days at 35 °C). Accelerated shelf-life testing is a useful tool that allows to speed up quality changes of a food product during storage and monitor them in a shorter time frame. Buvé et al. (2018) compared the effect of using different storage temperatures (20 °C, 28 °C, 35 °C and 42 °C) on color and anthocyanin stability of a pasteurized strawberry juice. They concluded that higher temperatures accelerated the color changes but did not induce other chemical reactions, therefore, confirming the applicability of the conditions used here to study the shelf life of strawberry-based products.

The change in total vitamin C content during 42 days of storage is shown in **Fig 4A**. For all processing conditions, the total vitamin C concentration, despite being different at the beginning, decreased markedly during storage yielding end values ranging from 2.5 to 4.6 mg/100 g FW in HB_25 °C and control_25 °C, respectively. As can be seen from **Fig 4A**, the degradation of vitamin C occurred in two clear phases, hence, a first-order biphasic model was selected to describe the vitamin C degradation. This biphasic behavior of vitamin C degradation in a strawberry matrix has already been described in the literature (Verbeyst et al., 2013) and it was shown to be influenced by the amount of oxygen present in

the sample. When oxygen is present in abundance the aerobic pathway is followed, here presumably between 0 to 4 days of storage (**Fig 4A**), in which AA is reversibly degraded to DHAA. Due to the instability of DHAA, this compound is further degraded to 2,3-diketogulonic acid (2,3-DKG) which further reacts to form other products such as xylosone, furfural and reductones (Shinoda et al., 2005; Corzo-Martínez et al., 2012). However, when oxygen is depleted or present in low concentrations, AA can be degraded through an anaerobic pathway (here presumably between 8 to 42 days of storage in **Fig 4A**). The anaerobic pathway results in the direct formation of 2,3-DKG from AA, which is further degraded into furfural. The degradation products of AA could either react with anthocyanins (Es-Safi et al., 2000) and cause the red color to fade, or form brown pigments in the presence of amino acids (Corzo-Martínez et al., 2012). The estimated reaction rate constants in the aerobic phase (k_{ae}), differed among the processing conditions, being the HB_25 °C the one with a significant lower rate constant (**Table 1**). These results are difficult to explain but the lower concentration of total vitamin C at the beginning of the storage for the HB_25 °C compared to the other two processing conditions could be an explanation. No differences in rate constants for the anaerobic phase were observed for the different processing conditions tested (**Table 1**). The DHAA content did not show a clear tendency but a scattered behavior during storage, independently of the treatment (data not shown). These observations are in accordance with Buvé et al. (2018) who reported no clear trend for DHAA during storage of strawberry juice at different temperatures (20-42 °C).

3.5 Changes in color-related attributes during storage

3.5.1 Total anthocyanin content and Polymeric color

The TAC decreased markedly after 42 days of storage, independently of the processing condition (**Fig 4B**). After storage, only about 2 mg anthocyanins/100 g FW were retained, meaning that 95% of the anthocyanins were degraded. These results support the idea that

storage has a major impact on anthocyanin stability (Oliveira and Almeida, 2014). Degradation of anthocyanins was best described by a first-order model ($R^2_{adj}>0.99$) which is in accordance with other authors (Buvé et al., 2018; Knebel et al., 2018). Despite not being fully understood, the degradation mechanism of anthocyanins during storage is attributed to enzymatic and non-enzymatic reactions (Oliveira et al., 2011). Linked to the enzymatic reactions, residual PPO activities after thermal processing were suggested to be responsible for color deterioration in strawberry products during storage (Gössinger et al., 2009a; Terefe et al., 2010; Holzwarth et al., 2012). Nevertheless, in this study, a complete PPO inactivation (i.e. the activity was below the limit of detection) was observed for all the samples after pasteurization, regardless of the processing history (**Fig 2A**) suggesting that role of PPO on color changes during storage would be limited. Moreover, despite the fact of different processing histories with higher PPO activities observed during the processing chain for control_25 °C compared to HB_25 °C and CB_4 °C, and the complete enzyme inactivation after pasteurization for all samples (**Fig 2A**), the rate of anthocyanin decay showed no significant differences (**Table 1**). It can be hypothesized that the contribution of secondary oxidation products (Sarni et al., 1995; Kader et al., 1999) formed upon PPO reactions during processing (e.g. mashing, waiting time) on anthocyanin degradation during storage, may be limited and non-enzymatic reactions should be considered as the main pathway for anthocyanin degradation. The latter can explain why no differences in TAC were observed for the different samples (**Fig 4B**) as it was assumed that anthocyanins were limitedly affected by the processing history.

It is known that AA can accelerate anthocyanin degradation through different mechanisms: (i) condensation reactions in position C4 of anthocyanins (Farr and Giusti, 2018) and/or (ii) oxidation by the degradation products of AA (i.e. H₂O₂ and furfural) (Es-Safi et al., 2000; Stebbins et al., 2016). In this study, we observed differences in the rate of vitamin C

degradation, but no differences were observed on the rate constants values for anthocyanin degradation. Hence, the contribution of AA on anthocyanin degradation seems limited and other reactions could play a major role. For example, condensation and polymerization reactions with other phenolic compounds (e.g. flavan-3-ols and proanthocyanidins) have been also suggested to result in anthocyanin degradation (Oliveira et al., 2011; Jiang et al., 2019). In this study, the decrease in anthocyanin content was accompanied by an increase in PC% values (**Fig 4C**) which means that monomeric anthocyanins were being polymerized. To the best of our knowledge, this is the first report presenting kinetic parameters for PC% formation in a strawberry-based product during storage. A first-order fractional conversion model best described ($R^2_{adj}>0.98$) the changes in PC%, which is in agreement with Turfan et al. (2011) findings in pomegranate juice. The increase in PC% throughout storage was generally similar in all samples.

3.5.2 CIELab values

The impact of storage on color stability of strawberry purees prepared under different processing conditions was evaluated using CIELab values and calculating derived parameters (C^* and h°) (**Fig 4D-F**). To better understand the color changes as a function of storage time, the data were modelled using a first-order model for h° values and a first-order fractional conversion model for a^* and C^* values. The estimated kinetic parameters are shown in **Table 1**. During storage, a^* and C^* values markedly decreased, achieving similar values in all samples at the end of the storage (**Fig 4D-E**). The decrease in a^* and C^* values during storage has been previously described in strawberry juice (Buvé et al., 2018) and puree (Howard et al., 2014) and could be attributed to anthocyanin degradation. The estimated k values for a^* and C^* values were not significantly different for samples treated differently, which is in agreement with the results in **Fig 4B** where no differences for the degradation rate of anthocyanins were found. Concomitant to a decrease in a^* and C^* , h° steadily increased

indicating a shift from red to orange-dark color (**Fig 4F**). A similar tendency for h^o was reported by Howard et al. (2014) in stored strawberry purees for 6 weeks at 25 °C. Based on the experimental data of the color measurements, it seemed that color changes occurring during storage appeared to be similar for all processing conditions based on the estimated reaction rate constants (k values). This suggests that PPO-catalyzed reactions occurring before pasteurization show a limited impact on color changes during shelf-life. Contrary to our results, Wrolstad et al. (1980) observed a higher browning rate and lower a^* values in an unblanched strawberry concentrate compared to a blanched concentrate after 8 weeks at 20 °C. However, in the latter study the concentrates were not pasteurized before storage. The similarity in rate constants for color changes during storage of pasteurized strawberry puree moreover suggests that the use of refrigerated temperatures (CB_4 °C) during processing does not improve the color and anthocyanin stability of the puree during shelf-life, as compared to the control.

4 Conclusion

The HB step (95 °C, 8 min) resulted in incomplete PPO inactivation and 10% of anthocyanin loss whereas AA remained unaffected. During the waiting time between crushing and pasteurization, anthocyanins and color remained unchanged but AA was oxidized to DHAA, which occurred faster at 25 °C than at 4 °C in the samples that were not pre-heated (HB). Pasteurization caused losses of anthocyanins and specially of vitamin C as well as a complete PPO inactivation, regardless from the treatment prior to pasteurization. Moreover, it has been clearly shown that partial enzyme inactivation prior to processing (HB step) or the use of refrigerated temperatures during the waiting time would not better retain the color and anthocyanins during puree processing. A first-order model (TAC and h^o), a first order biphasic model (total vitamin C) and a first-order fractional conversion model (PC%, a^* and

C^* values) were chosen to quantitatively describe the evolution of the different parameters. During storage for 42 days at 35 °C slower degradation of vitamin C was observed for the HB_25 °C. However, no differences were observed between the different processing conditions based on the estimated reaction rate constants for color and anthocyanin degradation. This suggests that the precursors formed upon enzymatic reactions (PPO) before pasteurization have a limited impact on color changes during shelf-life. Therefore, non-enzymatic reactions involving anthocyanin polymerization reactions and/or AA degradation reactions would play in major role on browning during shelf-life of strawberry puree and should be further investigated.

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6 Conflict of interest

Authors declare no conflict of interest

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FIGURE CAPTIONS

Figure 1. Overview of the experimental set-up with the processing steps for the three processing flows (Control_25 °C, Cold Break_4 °C and Hot Break_25 °C). The pasteurization value was $F_{95\text{ °C}}^{10\text{ °C}} = 1 \text{ min}$. Purees were stored at 35 °C for 42 days. Samples were collected at different time moments (indicated with a star symbol). Preparation and pasteurization were performed in triplicate.

Figure 2. (A) Residual polyphenol oxidase (PPO) activity, (B) total vitamin C, (C) ascorbic acid (AA) and (D) dehydroascorbic acid (DHAA) contents for the different strawberry purees (Control_25 °C, CB_4 °C, HB_25 °C). For the PPO assay, the enzyme activity was measured at 25 °C in all samples and additionally at 4 °C for CB_4 °C (4 °C) 3 h (grey coarse).

The results are the means and standard deviation (n=9) of samples, except for PPO (n=6). Different letters within one subfigure indicate significant differences ($P<0.05$) as determined by an ANOVA followed by Tukey HSD comparison test. nd = not detected

Figure 3. Changes in different color-related attributes of strawberry puree during the different processing steps (HB step, 3 h waiting time and pasteurization). (A) Total anthocyanin content (TAC), (B) polymeric color % (PC%), (C) redness (a^*) value, (D) chroma (C^*) value, (E) lightness (L^*) value and (F) hue angle (h°) value.

The results are means and standard deviation (n=9) of samples. Different letters within the same subfigure are significantly different ($P<0.05$) as determined by an ANOVA followed by Tukey HSD comparison test.

Figure 4. Changes in (A) total vitamin C (B) total anthocyanin content (TAC), (C) polymeric color % (PC%), (D) redness (a^*) value, (E) chroma (C^*) value and (F) hue angle (h°) value during 42 days storage at 35 °C in purees prepared under different processing conditions: control_25 °C (circle), CB_4 °C (triangle) and HB_25 °C (square). Different symbols represent the experimental data (means and standard deviation of n=3) and the solid lines represent the predicted behavior.

Figure 1.

Control_25 °C

Cold Break_4 °C

Hot Break_25 °C

770 **Figure 2.**

785 **Figure 3**

Table 1. Estimated rate constants (k) at 35 °C ($\pm$ standard error of regression) for each attribute and processing condition. Estimated kinetic parameters were obtained following Eq. (7), (8) and (9) depending on the attribute.

Different letters within the same quality attribute mean significant differences ($P<0.05$) between the k -values for each processing condition.

Quality Attribute	C ₀	C _∞	k(days ⁻¹)		R ² _{adj}	SEE
First order fractional conversion model						
a* (redness)						
Control_25 °C	38.33±0.19	17.63±0.29	0.0725±0.003 ^a		0.997	0.392
Cold Break_4 °C	37.66±0.23	17.73±0.35	0.0750±0.004 ^a		0.995	0.491
Hot Break_25 °C	37.79±0.16	17.98±0.20	0.0830±0.002 ^a		0.998	0.332
C* (chroma)						
Control_25 °C	44.68±0.28	27.30±0.29	0.102±0.005 ^a		0.992	0.560
Cold Break_4 °C	43.86±0.36	27.17±0.35	0.109±0.008 ^a		0.987	0.711
Hot Break_25 °C	43.86±0.30	26.50±0.30	0.106±0.006 ^a		0.991	0.601
Polymeric color (%)						
Control_25 °C	19.95±0.77	73.98±1.61	0.060±0.005 ^b		0.992	1.672
Cold Break_4 °C	17.15±0.75	75.68±2.27	0.049±0.004 ^c		0.992	1.661
Hot Break_25 °C	12.96±1.08	69.30±1.41	0.084±0.007 ^a		0.988	2.243
First order model						
h° (hue angle)						
Control_25 °C	30.95±0.38	-	0.011±0.0004 ^a		0.990	1.310
Cold Break_4 °C	30.70±0.39	-	0.011±0.0004 ^a		0.999	1.353
Hot Break_25 °C	31.28±0.46	-	0.010±0.0005 ^a		0.998	1.597
Total Anthocyanin Content (mg/100 g FW)						
Control_25 °C	45.30±0.91	-	0.105±0.005 ^a		0.995	1.887
Cold Break_4 °C	46.20±0.91	-	0.106±0.005 ^a		0.995	1.910
Hot Break_25 °C	41.04±0.78	-	0.106±0.005 ^a		0.995	1.636
Biphasic model						
Total Vitamin C content (mg/100 g FW)						
	C _{ae}	C _{an}	k _{ae} (days ⁻¹)	k _{an} (days ⁻¹)		
Control_25 °C	21.31±0.53	12.98±6.95	0.68±0.18 ^a	0.03±0.003 ^a	0.994	0.740
Cold Break_4 °C	23.55±0.21	12.14±3.33	0.60±0.05 ^a	0.03±0.001 ^a	0.999	0.345
Hot Break_25 °C	18.02±4.4	5.22±1.19	0.27±0.04 ^b	0.02±0.01 ^a	0.994	0.748

Supplementary Material

Figure A1. Temperature profile during the pasteurization with a target process value of $F_{95^{\circ}\text{C}}^{10^{\circ}\text{C}} = 1 \text{ min}$. A thermal process in the retort (dashed lines) and the temperature in the coldest spot of the sample (grey line) of control_25 °C.

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839 **Figure A2.** Temperature profile during pre-heat treatment (95 °C, 8 min) in fresh
840 strawberries. Temperature was measured in the core of two different fruits.

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