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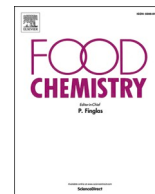
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# Comparing the impact of conventional and non-conventional processing technologies on water-soluble vitamins and color in strawberry nectar – a pilot scale study

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## ABSTRACT

A comprehensive comparison was conducted on the effect of conventional thermal processing (TT), high-pressure processing (HP), pulse electric field (PF), and ohmic heating (OH) on water-soluble vitamins and color retention in strawberry nectar. The ascorbic acid (AA) content increased by 15- and 9-fold after TT and OH treatment, respectively, due to rupturing of cells under heat stress and release of intracellular AA. Dehydroascorbic acid (DHA) content did not change considerably after TT and PF treatment but significantly decreased after HP and OH treatment. TT treatment offered the highest total vitamin C retention. The B vitamins remained largely unchanged after processing, with the highest loss of 34 % for riboflavin in OH-treated samples. All the technologies resulted in similar color retention after processing. The study concludes with a standardized comparison of mainstream preservation technologies using pilot-scale equipment. Such an approach significantly increases the applicability of the results presented in the study.

## 1. Introduction

Several alternatives to conventional thermal processing (TT) have been developed to stabilize food products, but only a few are utilized commercially. This is due to hurdles encountered during up-scaling, for example, the high cost of production (Rastogi et al., 2007) and consumer concern about their effect on human health (Junqueira-Gonçaves et al., 2011). The fruit juice and beverage industry has been less resistant to adopting new processing technologies than other food sectors (Martins et al., 2019). High-pressure processing (HP), ohmic heating (OH), and pulsed electric field (PF) have been successfully used for preservation of

fruit juice and beverages at commercial scale and continue to grow in use in the global food market (Alkanan et al., 2021; Morales-de La Peña et al., 2016).

Vitamins are classified as essential micro-nutrients, required for several different human physiological functions (Zhang et al., 2018). Fruit juices and beverages are a good source of water-soluble vitamins; therefore, vitamin retention in these products is a critical quality attribute (Mitchell et al., 2020). Non-conventional food processing technologies are believed to ensure better vitamin retention in comparison to TT treatment mainly due to short treatment time and low operating temperatures (Dhakal et al., 2018; Morales-de La Peña et al., 2016; Raj

*Abbreviations:* B5, pantothenic acid; B3-A, nicotinic acid; B3-M, nicotinamide; B2, riboflavin; B1, thiamine; B6, pyridoxine; TT, conventional thermal treatment; OH, ohmic heating; HP, high-pressure processing; PF, pulse electric field; AA, ascorbic acid; DHA, dehydroascorbic acid; AF, acceptance factor; PPE, polyphenol-degrading enzymes; AAO, ascorbic acid oxidase..

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et al., 2019; Xu et al., 2018). Recent review studies comparing the impact of TT, HP, OH, and PF treatment on the quality of fruit juices and beverages, however, demonstrate that the knowledge on the topic is fragmentary and, in several cases, contradictory (Houška et al., 2022; Petruzzi et al., 2017). While the literature generally suggests that ascorbic acid (AA) is thermally labile and that alternatives to thermal processing are better for AA retention, some reports contradict this belief (Landl et al., 2010; Lima et al., 2010; Mercali et al., 2014; Timmermans et al., 2022). Certain studies have shown that thermal treatment (TT) is as effective as alternative techniques in retaining AA (Leizeron & Shimoni, 2005; Tumpanuvat & Jittanit, 2012; Wibowo et al., 2019). Zheng et al. (2014) even found higher AA retention in TT-treated litchi juice compared to HP-treated juice. The variation in results reported in the literature can be attributed to differences in the stability of ascorbic acid (AA) across different matrices, which is influenced by factors such as pH, water activity, and the presence of endogenous components like enzymes and bioactive compounds (Capuano et al., 2017; Herbig & Renard, 2017; Mercali et al., 2014). Another reason for the varied results is the lack of standardization in TT treatment methods. The common use of static water baths in studies (Aaby et al., 2018; Achir et al., 2016; Sánchez-Vega et al., 2009; Wu et al., 2021) is not optimal for heating low-viscosity fluids like fruit juices due to high thermal resistance (Greiby et al., 2017; Lalwani et al., 2021). A more realistic setup involves using tube and plate heat exchangers, commonly used in the industry for their rapid heat transfer rate (Huang et al., 2020).

Additionally, the discussion on comparing the impact of preservation technologies on vitamin retention in fruit juice and beverages is mainly limited to AA alone. Little information is available on the effect of TT treatment or other preservation technologies on dehydroascorbic acid (DHA). DHA is produced by spontaneous oxidation of AA in fruit juices and beverages and carries the same biological activity as AA (Spínola et al., 2012). Generally, DHA determination is ignored when estimating the total vitamin C content of a sample since it was reported to be present in a much lower proportion than AA (Lee & Kader, 2000). Recent studies, however, report that the DHA content can make up to 60 % of the total vitamin C content in fruits and fruit-derived products (Fenoll et al., 2011; Landl et al., 2010). This corroborates the theory that the determination of only AA content can lead to a significant underestimation of total vitamin C content. Like DHA, knowledge is scarce regarding the influence of different processing technologies on B vitamin content in fruit juices and beverages. Most studies discuss the stability of B vitamins in fruits or fruit-derived products when subjected to different drying techniques (Batu & Kadakal, 2021; Çakmak et al., 2020; Fernandes et al., 2015) or during their storage under various conditions (Gutzeit et al., 2007).

Color is another important quality attribute of processed fruit juices and beverages, as it directly correlates to consumer acceptance (Gössinger, Mayer, et al., 2009). One of the main reasons for the color deterioration of fruit juices and beverages is the high residual activity of polyphenol- and anthocyanin-degrading enzymes (Teribia et al., 2021). While TT treatment can ensure better color stability of processed fruit juices and beverages due to inactivation of enzymes, it can also potentially result in significant degradation of color quality due to accelerated non-enzymatic browning (Achir et al., 2016). Non-conventional preservation technologies such as HP, OH, and PF treatments have been reported to result in better color retention after processing, in comparison to TT treatment, due to shorter treatment time and lower heat load (Achir et al., 2016; Houška et al., 2022; Timmermans et al., 2022). However, some studies report no significant difference between the color attribute of TT-treated and non-conventionally processed juices (Doan et al., 2023; Khuenpet & Jittanit, 2020; Mannozi et al., 2019). Studies also report better color retention with TT treatment compared to non-conventional techniques (Aganovic et al., 2015; Song et al., 2023; Wibowo et al., 2019). As with vitamin degradation, the difference in color retention results can be attributed to differences in studies' conditions, such as the type of matrix and processing equipment. While TT

and OH treatments have been used typically to stabilize nectars and juices, PF and HP treatments are mainly used to extend shelf life under cold storage. In the former comparisons reported in the literature, the processing conditions for the different treatments were not normalized, i.e., resulting in a similar log reduction of a reference microorganism (Ağcam et al., 2019; Houška et al., 2022; Petruzzi et al., 2017). To ensure fair and reliable characterization of the effects of different preservation technologies on the quality of fruit juices and beverages, it is essential to perform a comparison under comparable conditions.

The presented study aimed to systematically and comprehensively compare the effect of different commercially relevant preservation technologies (TT, OH, PF, and HP) on water-soluble vitamins and color retention in strawberry nectar. Strawberry nectar is examined in the study because it is a rich source of water-soluble vitamins (Giampieri et al., 2012). This allows for a comprehensive analysis of the various water-soluble vitamins naturally present in fruit-derived products. Additionally, the color of strawberry nectar is highly susceptible to processing, and color degradation directly influences consumer acceptance; therefore, it can serve as a representative for fruit juices and beverages with low color stability (Gössinger, Mayer, et al., 2009). The water-soluble vitamins and color retention in differently treated samples were also investigated as a function of storage time. The comparison will help support the theory that emerging thermal and non-thermal technologies are better for color and vitamin retention in processed fruit juices and beverages than TT treatment. The study uniquely offers a comparison of TT treatment and three mainstream non-conventional processing techniques in a single study. This study is also the first to compare the influence of preservation technologies on all the major water-soluble vitamins naturally present in fruit-derived extracts. Additionally, this is the first study of its kind where the preservation technologies have been compared on a pilot-scale level using normalized processing conditions i.e. similar log reduction of a reference microorganism. Moreover, using the same raw material for all trials ensures no matrix effects influence the results. These features of the study significantly increase the applicability and relevance of the results for the fruit juice and beverage industry.

## 2. Materials and methods

### 2.1. Chemicals

The chemicals were purchased from Sigma-Aldrich Chemie GmbH (Germany), Chemsolute (Germany), Honeywell (Germany), J.T.Baker (Poland), Carl Roth GmbH (Germany), Carlo Erba Reagents (France), Merck KGaA (Germany): L-dehydroascorbic acid (Aldrich 261,556), ammonium acetate (Erba 418,781), L-ascorbic acid (Baker 1018), ethylenedinitrilotetraacetic acid EDTA (Aldrich 108,418), acetic acid (Chemsolute 2289), ascorbic acid oxidase (Roth 10,736,619,001), pyridoxine (Aldrich P5669), cyanocobalamin (Roth T915.2), riboflavin (Aldrich R4500), thiamine hydrochloride (Aldrich T4625), biotin (Aldrich B4639), folic acid (Aldrich F7876), d-pantothenic acid hemicalcium salt (Aldrich 21,210), nicotinamide (Aldrich 72,340), ammonium formate (Erba 419,741), nicotinic acid (EMD 569470), formic acid (Chemsolute 2694), methanol for LC-MS (Chemsolute 1428), acetonitrile for LC-MS (Chemsolute 2697). Ultra-pure 18.2 MΩ Millipore water from Milli-Q Plus 185 (Germany) was used in the experiments.

### 2.2. Strawberry nectar preparation

The nectar was prepared from a single batch of fruit sourced from a single supplier to ensure homogeneity in the raw material used for the pilot scale trials. This eliminates confounding factors, such as differences in endogenous matrix components that could affect the results. The strawberry puree was sourced from SVZ, Spain. Due to the large quantities needed for the OH and TT treatment trials, it was not possible to obtain strawberry puree from a single variety of strawberries due to

limited availability. Therefore, the puree came from a blend of three different strawberry varieties, which is standard in the industrial production of strawberry nectar. The lot of strawberry puree was divided into required fractions and transported under frozen storage to the respective industrial partners in the project for pilot scale trials. The transport was made in 18 kg leakproof cardboard boxes. The boxes of strawberries were thawed at room temperature until they were soft enough to be blended. The strawberry nectar was prepared after blending 40 % strawberry puree with water. Sucrose and citric acid were added to adjust the soluble solids and titratable acidity to 12° and 5 g/kg (calculated as citric acid, pH 8.1), respectively. The strawberry nectar was prepared fresh before the respective trials.

### 2.3. Pilot scale trials

Pilot scale trials were conducted using pilot plants available at the respective facilities of the industrial partners in the project. The TT treatment trials were undertaken at CFT in Parma, Italy. The HP treatment trials were undertaken at HPP Italia in Parma, Italy. The OH treatment trials were undertaken in CTCPA in Avignon, France. PF treatment trials were undertaken at ELEA in Quakenbrück, Germany. The processing conditions for the respective trials are summarized in Table 1. The processing conditions for TT and OH treatment were determined using the thermal resistance parameters for the reference microbe, which were established under laboratory conditions (Geđas et al., 2024) and then validated on an industrial scale for both processing techniques. The conditions for PF and HP were initially estimated using available literature (Darvishi et al., 2021; Mendes-Oliveira et al., 2020; Usaga et al., 2021) and then further optimized using trial-and-error approach using a pilot-scale apparatus to achieve a 5-log reduction of the reference microorganism in strawberry nectar. Considering the large quantity of puree needed, the chosen processing conditions for TT and OH treatment were validated using a surrogate matrix for the 5-log reduction condition at the pilot scale. The surrogate matrix was a solution of sugar with water, and soluble solids and titratable acidity were adjusted to 12° and 5 g/kg (calculated as citric acid, pH 8.1), respectively, using sucrose and citric acid. A bag of untreated strawberry nectar was also collected for each processing technology on the respective trial day to serve as a control for analysis. The samples were kept in frozen storage (− 18 °C) until further transport.

### 2.4. Sample preparation

A set of samples for each preservation technique consisted of four bags, including one bag of untreated nectar collected on the day of trial and three bags of treated nectar to analyze on days 0, 30, and 60. The strawberry nectar samples were immediately frozen after production. They were distributed by commercial transport at −18 °C to partner organizations for analysis. The sample bags were thawed in a 30 °C

**Table 1**

The summary of processing conditions for the processing technologies. TT: Conventional thermal treatment, HP: High-pressure processing, PF: Pulse electric field, and OH: Ohmic heating.

Processing technique	Processing parameters
TT	Temperature: 72 °C Holding time: 117 s
HP	Applied pressure: 600 MPa Holding time: 5 min
PF	Field strength: 20 kV/cm Specific Energy: 100 kJ/Kg Preheating Temperature: 40 °C Treatment time: 109 μs
OH	Temperature: 72 °C Holding time: 117 s Frequency: 25 kHz Field strength: 80 V/cm

water bath for around 2 h. The day of the thawing of samples marked the day 0 for analysis. The untreated strawberry nectar and day 0 samples were analyzed on the day of thawing. The samples for 30- and 60-day analyses were kept at 4 °C until the day of analysis.

### 2.5. Analysis of vitamins

The B vitamins in the fruit juice samples were analyzed using an HPLC-MS/MS multivitamin analysis method (Zia et al., 2023a). The standard solutions and the dilution of nectar samples were prepared using a 0.1 % (v/v) aqueous formic acid solution. The working standard solutions for B vitamins were prepared fresh on the day of analysis using aliquoted stock solutions that were stored at −20 °C (stable for six months). A multivitamin juice (Art No. 1121018–011) provided by the reference material laboratory of Germany (DRRR GmbH) served as quality control for the analysis of B vitamins. The AA and DHA in the samples were analyzed simultaneously using an HPLC-MS/MS method (Zia et al., 2023b). The standard solution preparation and dilution of samples was done using 10 mM ammonium acetate buffer in water (pH = 3) with 0.05 % (w/v) of EDTA. The stock solutions of DHA and AA were prepared fresh on the day of analysis. Two different reference materials were used as quality control for AA analysis: PHR1008 from Sigma-Aldrich, Germany, and multivitamin juice (Art No. 1121018–011) from DRRR GmbH, Germany. Unfortunately, no reference material is commercially available for DHA. Therefore, an in-house reference standard solution of DHA was prepared after enzymatic oxidation of AA standard solution, as described in Zia et al. (2023b), and was used as quality control for DHA analysis. The treated strawberry nectar samples were diluted to the required dilution factor and filtered into HPLC vials using CHROMAFIL PET-20/15 MS filters, 0.2 μm (Macherey-Nagel, Germany) for analysis. The preparation of working standard solutions of vitamins and the dilution of samples was done under subdued light and with amber glassware to prevent any deterioration of vitamins due to light. The ion chromatograms were analyzed using the SCIEX OS software (2.2.6.59781).

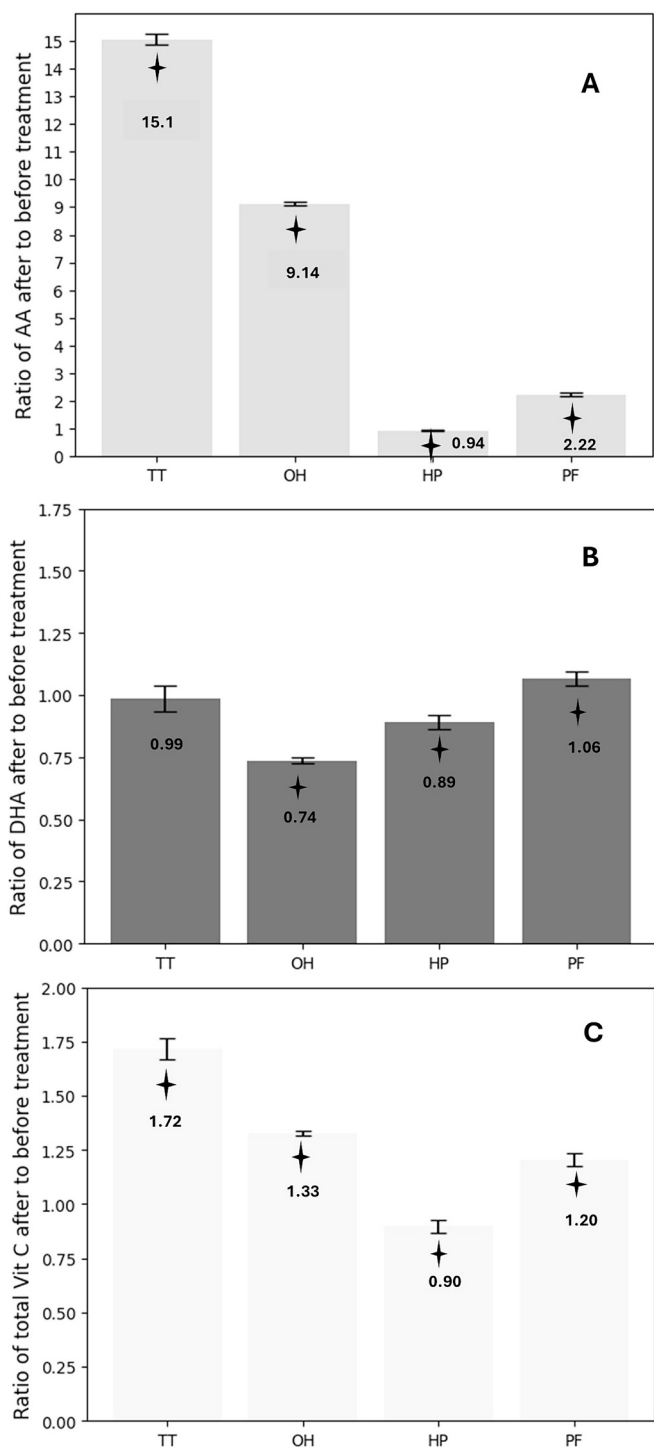
### 2.6. Analysis of color

CIELAB-system color components L\* (lightness), a\* (red-green), and b\* (yellow-blue) were measured by a Minolta CM-5 spectrophotometer (spectrophotometric method, D65, 30 mm 10°, reflection measurement, gloss excluded, Minolta, Osaka, Japan). C\* (Chroma) and h° (hue angle) were calculated as previously reported (Kammerer et al., 2006). The total color difference ΔE was calculated and the color change was categorized as not noticeable (0–0.5), slightly perceptible (0.5–1.5), evident (1.5–3.0), clearly visible (3.0–6.0), or big color difference (6.0–12.0) (Lacey et al., 2023). An acceptance factor (AF = a\*/h), derived from CIELAB color components, was also determined for the samples to get further insights on any perceivable color changes (Gössinger, Moritz, et al., 2009). AF can quantify the likelihood of consumers accepting the color of nectar and can be used to track the degradation of nectar's color after processing and during storage (Gössinger, Moritz, et al., 2009). Nectars with AF > 0.7 were considered excellent in color, and AF < 0.4 were considered unacceptable (Murray et al., 2023).

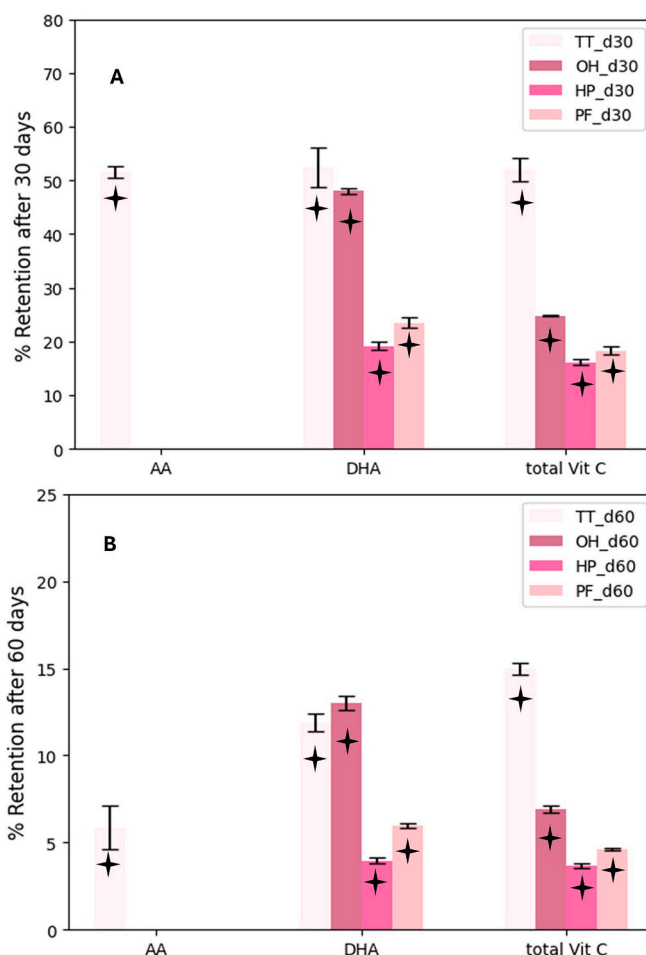
### 2.7. Data analysis

The samples were analyzed in triplicate (n = 3). The acceptability limit (λ) for the accuracy of quality controls used for vitamin analysis was set at ±25 % - a typical value used when working with the multi-component analysis method (Jitaru et al., 2016). The significance level (α) was set at 5 %. The IBM SPSS 26 (Statistical Package for the Social Sciences) was used to perform ANOVA, followed by post hoc Tukey's test to establish significant differences between the color components and other calculated parameters related to the color quality of different

treatments. Python 3.10 was used to statistically test data related to vitamins and produce figures to present data for vitamins. The summary of the statistical tests conducted for data related to vitamins are presented in Appendix A. The equations used to report factor change, percentage change, and associated uncertainty in Fig. 1–3 are provided in



**Fig. 1.** Change in the content of AA (A), DHA (B), and total vitamin C content (C) in strawberry nectar after treatment. The y-axis presents the ratio of the concentration of the respective analyte after the treatment to that present before the treatment. The star symbol shows a significant difference in the content between treated and untreated nectar ( $n = 3$ ,  $p < 0.05$ ). AA: ascorbic acid, DHA: dehydroascorbic acid; total vitamin C = AA+DHA; TT: conventional thermal treatment, OH: ohmic heating, HP: high-pressure processing, PF: pulse electric field.



**Fig. 2.** Change in the content of ascorbic acid (AA), dehydroascorbic acid (DHA), and total vitamin C content (AA+DHA) in treated strawberry after 30 days (A) and 60 days (B) of storage at 4 °C. The star symbol shows that there was a significant decrease in the content of analytes in the treated samples ( $n = 3$ ,  $p < 0.05$ ). The % retention after 30 days (A) and 60 days (B) corresponds to the ratio of analytes' content on day 30 and day 60, respectively, to the analytes' content on day 0 in treated strawberry nectar. TT: conventional thermal treatment, OH: ohmic heating, HP: high-pressure processing, PF: pulse electric field.

Appendix C.

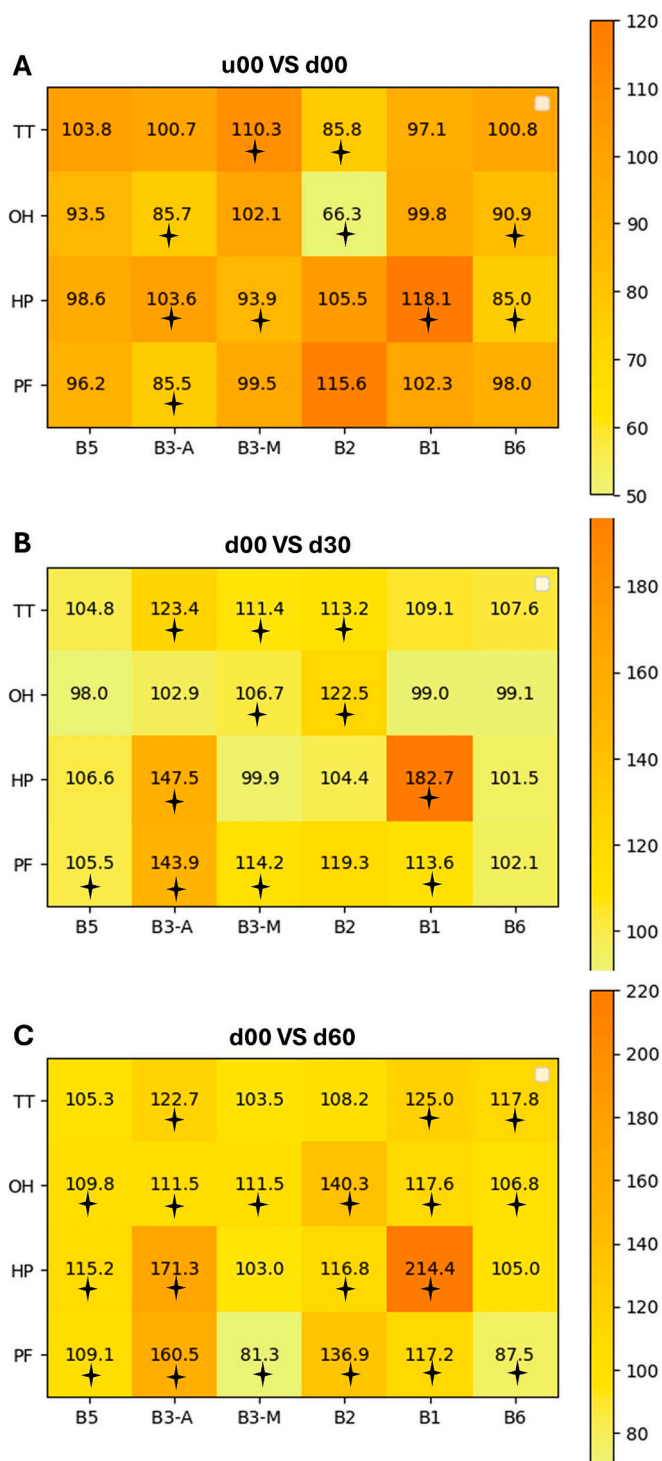
### 3. Results and discussion

#### 3.1. Stability of vitamin C during treatment

The true content of total vitamin C in a sample is reflected by the combined amount of AA and DHA (Zia et al., 2023b). Therefore, both AA and DHA were analyzed in this study to fully understand the influence of different preservation technologies on total vitamin C content.

##### 3.1.1. Ascorbic acid

The AA content in strawberry nectar decreased by a slight margin of 6 % after HP treatment (Fig. 1A). The HP treatment has been reported to have little to no effect on the covalent bonds of bioactive compounds, i. e., vitamins (Houška et al., 2022). The content of AA did not change when grapefruit juice was subjected to HP treatment at 402 MPa for 3 min (Uckoo et al., 2013). Similarly, HP treatment at 500 MPa for 10 min of pineapple juice (Wu et al., 2021) and kiwi fruit juice (Xu et al., 2018) did not change AA content. In a study by Aaby et al. (2018), HP treatment of strawberry puree at 600 MPa for 3 min did not change AA



**Fig. 3.** Change in the content of B vitamins in strawberry nectar after treatment (A) and after storage of treated strawberry nectar at 4 °C for 30 days (B) and 60 days (C). The change in the vitamin B content is reported as % retention as explained in appendix C. The star symbol indicates the statistically significant difference ( $n = 3$ ,  $p < 0.05$ ). B5: pantothenic acid, B3-A: nicotinic acid, B3-M: nicotinamide, B2: riboflavin, B1: thiamine, B6: pyridoxine, TT: conventional thermal treatment, OH: ohmic heating, HP: high-pressure processing, PF: pulse electric field, u00: untreated nectar, d00: treated nectar after treatment, d30 and d60: treated nectar after 30 and 60 days of storage, respectively.

content. In another study, the treatment of strawberry puree at 600 MPa for 15 min resulted in a significant decrease in AA content (Patras et al., 2009). These results showcase that apart from the magnitude of pressure, processing time is also a crucial parameter to consider when studying the retention of vitamins in fruit extracts.

The AA content in strawberry nectar increased by around 2.2-fold after PF treatment (Fig. 1A). As in the case of HP, PF treatment has been demonstrated to have little to no effect on bioactive compounds like vitamins (Soliva-Fortuny et al., 2009). The increase in AA after PF treatment can be explained by electroporation. PF treatment alters the transmembrane potential, and when this change exceeds a certain threshold, it forms hydrophilic pores, resulting in cell membrane permeabilization – a phenomenon called electroporation (Demir et al., 2023). The increased porosity of the membrane can facilitate the release of intracellular substances (Weber & Larsen, 2017; Zeng & Zhang, 2019). The AA content in grape juice increased following PF treatment, and the effect could be attributed to electroporation (Leong et al., 2016). Odriozola-Serrano et al. (2008) reported no significant change in AA content after strawberry juice was subjected to PF treatment, which was not corroborated by this study. The difference in results could be because the authors used filtered strawberry juice rather than the unfiltered nectar used in this study. The high cellular matrix content in unfiltered nectar, entrapping a considerable amount of AA, may have contributed to the notable increase in AA content after PF treatment due to the electroporation effect. AA has been observed to behave differently in strawberry puree compared to filtered strawberry juice when undergoing processing (Aaby et al., 2018). Additionally, Odriozola-Serrano et al., 2008) used significantly higher electric field strength (35 KV/cm) and longer treatment time (1700  $\mu$ s) than the parameters used in the current study, which resulted in a higher degradation of AA that countered the release of intracellular AA via electroporation. The use of high electric field strength and longer treatment times have been reported to contribute to increased AA degradation (Ağçam et al., 2016; Cortés et al., 2007; Sánchez-Vega et al., 2015).

A 15-fold and 9-fold increase in AA content was found for TT- and OH-treated samples, respectively (Fig. 1A). Khuenpet and Jittanit (2020) reported a significant increase in AA content after TT and OH treatment of Madan fruit juice and concentrate at 95 °C for 15 s. A significant increase in total vitamin C content (combined amount of AA and DHA) was reported upon TT treatment of strawberry puree at 85 °C for 2 min (Aaby et al., 2018). The authors of these studies, however, did not provide a plausible explanation or underlying mechanism behind the increase of total vitamin C upon heating. The increase in AA can potentially be associated with the rupturing of cells under heat stress. The heat generated inside the cells at high temperatures increases water vapors' pressure, causing the cells to swell to the point of rupturing the cell wall and releasing intracellular compounds (Carpentieri et al., 2021). Vitamin C is found in different cell compartments, such as the cell wall, chloroplast, and plastids (Aguilar et al., 2017; Paciolla et al., 2019; Xu et al., 2018). It can be expected that the AA is released from the cellular components upon heating the strawberry nectar due to the potential rupturing of intact cells. Disruption of cells and subsequent release of intracellular bioactive compounds like AA is also possible during HP treatment, leading to an overall increase in AA content (Kurek et al., 2022). However, in this study, total AA content decreased after HP treatment. This could be due to the high residual activity of enzymes like ascorbic acid oxidases (AAO) (Muñoz et al., 2023) and polyphenol degrading enzymes (PPE) present in the strawberry fruit (Teribia et al., 2021). While AAO directly causes oxidative loss of AA, PPE causes significant degradation of polyphenolic compounds and indirectly influences the stability of AA since polyphenolic compounds are known to provide stability to AA against degradation (Herbig & Renard, 2017; Miller & Rice-Evans, 1997). The release of AA due to the rupturing of cells under high pressure is countered by the higher rate of degradation of AA due to higher residual enzyme activity, potentially resulting in a net decrease in AA content in HP-treated samples.

### 3.1.2. Dehydroascorbic acid and total vitamin C

The changes in DHA content in strawberry nectar due to processing are presented in Fig. 1B. Knowledge of the effect of preservation technologies on the DHA content in fruit juices is scarce in the literature, making it challenging to discuss and compare the acquired results. Since DHA is more unstable than AA (Deutsch, 2000; Mieszczakowska-Frać et al., 2021), a significant reduction in DHA content due to processing was expected. The content of DHA decreased significantly by approximately 26 % and 11 % following OH and HP treatments, respectively. The higher degree of degradation for DHA during OH treatment can be attributed to potential electrochemical degradation (Assiry et al., 2006; Sánchez-Vega et al., 2015). The DHA content increased significantly by 6 % following PF treatment. As explained in section 3.1.1, the electro-oxidation effect could have led to the release of DHA inside the intact cells. Another plausible explanation for the increase in DHA content could be the increase in temperature during PF treatment that led to increased oxidation of AA to DHA (Timmermans et al., 2022). Interestingly, the DHA content did not change following TT treatment. It is important to highlight that the DHA results presented in the study do not necessarily depict the stability of DHA against the different preservation technologies studied. There is spontaneous oxidation of AA to DHA in food samples, and the conversion rate accelerates at elevated temperatures (Fenoll et al., 2011; Timmermans et al., 2022). The new DHA formation continuously compensates for the degradation of DHA due to processing. This also possibly explains why this study reported no significant change in AA content for TT-treated strawberry nectar. Considering changes in total vitamin C content in the samples (Fig. 1C), TT-treated samples performed the best in terms of Vitamin C retention right after treatment with a significant 72 % increase in comparison to the untreated nectar, followed by approximately 30 % and 20 % significant increases in total vitamin C for OH- and PF-treated samples, respectively. The high retention of total vitamin C in TT-, OH- and PF-treated samples is attributed to the significant increase in AA content, as explained in section 3.1.1. The HP treatment had the worst vitamin C retention, with a significant 25 % decrease observed for HP-treated samples. The higher degree of vitamin C degradation with HP treatment is because of high residual enzyme activity, as described in section 3.1.1. The concentrations (mg/L) for AA, DHA, and total vitamin C in the untreated and treated strawberry nectar samples are presented in Table B.1 in Appendix B. The factor changes and the associated estimated uncertainty, reported in Fig. 1, are presented in Table B.2 in Appendix B.

### 3.2. Stability of vitamin C during storage

After 30 days of storage at 4 °C, the HP-, OH- and PF-treated samples had lost all the AA (Fig. 2A). The TT-treated samples lost around 50 % of the AA in the first 30 days, and the AA loss increased to approximately 94 % by the end of 60 days of storage (Fig. 2B). The DHA content and the total vitamin C content also decreased during storage. The highest losses were in HP-treated samples, followed by PF, OH, and TT treatment (Fig. 2). As mentioned earlier in section 3.1.1, the HP treatment is less effective against inactivation of enzymes than other preservation technologies like TT, OH, and PF treatment (Landl et al., 2010; Wibowo et al., 2019). The high residual enzyme activity in HP-treated samples is the leading cause of the instability of vitamin C during storage (Li et al., 2021). At the end of 8 weeks of storage period at 4 °C, TT-treated samples lost 75 % of total vitamin C, and losses were in the range of 93–96 % for other technologies. The concentrations (mg/L) for AA, DHA, and total vitamin C in the treated strawberry nectar samples on days 30 and 60 of storage are presented in Table B.1. in Appendix B. The % retention and the associated uncertainty, reported in Fig. 2, are presented in Table B.3. in Appendix B.

### 3.3. Stability of B vitamins during treatment

Fig. 3A summarizes the results of B vitamins analysis in differently

treated strawberry nectar samples. Out of the nine major B vitamins (Zia et al., 2023a), biotin (B7), folic acid (B9), and cyanocobalamin (B12) were not present in the strawberry nectar. This was expected since these vitamins are typically absent in food material of plant origin, like fruit juices, and are intentionally added for fortification (Gregory, 2012; Van Den Oever & Mayer, 2022). Pantothenic acid (B5) was the most stable vitamin as its content did not change significantly after processing for all of the preservation technologies. Like B5, pyridoxine (B6) was stable against TT and PF treatment, with no significant change reported after treatment. However, the B6 content decreased by 9 % and 15 % for OH- and HP-treated samples, respectively. B1 was also stable against preservation technologies with no significant reduction in content after TT, OH, and PF treatment. The HP-treated samples reported a significant 18.1 % increase in the content of B1 after treatment. Similarly, nicotinic acid (B3-A) content also increased significantly in HP-treated samples by 3.6 %. While the content of B3-A remained unchanged after TT treatment, it decreased significantly by around 15 % for OH and PF treatment. Nicotinamide (B3-M) also demonstrated high stability against all treatments except HP. B3-M decreased significantly by around 6 % in HP-treated samples. TT-treated strawberry nectar demonstrated a significant 10 % increase in the content of B3-M. Riboflavin (B2) demonstrated the least thermal stability with a significant decrease of 14 % and 34 % after TT and OH treatment, respectively. B2 was stable against HP and PF as no significant changes were reported.

As the literature is limited on the influence of preservation technologies on the stability of B vitamins in fruit juices, it is hard to compare the findings of the current study. There have been some reports in the literature on the thermal stability of B vitamins in fruit extracts. For example, B5 and B3-A showed only a slight degradation in bilimbi fruit extract when heated at 90 and 100 °C for a few minutes (Muhamad et al., 2015). Contrary to the results in the current study, B1 was found to be sensitive to heat, with a 30 % reduction in TT-treated bilimbi fruit extract (Muhamad et al., 2015). In a study by Kadakal et al. (2017), B2 in rosehip nectar exhibited high stability towards TT treatment. The difference in results could be due to differences in matrices (Capuano et al., 2017) and the processing conditions. As mentioned in the case of AA in section 3.1.1, the increase in the content of certain B vitamins as a result of processing can be attributed to the release of bound vitamins due to the rupturing of cells, which increases the total content of free vitamin that is later quantified by the method. Overall, the highest retention of the B vitamins was seen for HP- and TT-treated samples. The OH-treated samples demonstrated the least retention of B vitamins after treatment. The higher reduction of B vitamins in OH-treated samples could be attributed to electrochemical degradation effects as reported for AA in the literature (Assiry et al., 2006; Sánchez-Vega et al., 2015). The concentrations (mg/L) for B vitamins in the untreated and treated strawberry nectar samples are presented in Table B.4 in Appendix B.

### 3.4. Stability of B vitamins during storage

The results of B vitamin retention in differently treated strawberry nectars after 30 and 60 days of storage at 4 °C are presented in Fig. 3B and C, respectively. After 30 days of storage, there was no significant reduction in the content of B vitamins in all the differently treated strawberry nectar. The content of B6 did not change significantly in all the differently treated strawberry nectar after 30 days of storage. Similarly, the content of B5 did not change significantly for all the differently treated strawberry nectar except for the PF-treated, which had a 5.5 % increase in the content after the first 30 days of storage. The content of B3-A, B3-M, and B1 also increased significantly by 43.9 %, 14.2 %, and 13.6 %, respectively, in the PF-treated samples after the first 30 days of storage. The content of the majority of B vitamins remained unchanged for the first 30 days of storage for HP-treated samples with a significantly large increase of 47.5 % and 82.7 % in the content of B3-A and B1, respectively. At the end of 8 weeks of storage at 4 °C (Fig. 3C), the only significant reduction observed was in the case of B6 and B3-M in

PF-treated samples, with 18.7 % and 12.5 % decrease, respectively. The level of B5 in TT-treated samples and B6 in HP-treated samples remained significantly unchanged during the storage time of eight weeks at 4 °C. In most cases, the B vitamin content continued to rise in treated strawberry nectar samples during the final four weeks of storage. The most notable increases were seen in HP-treated samples, with B3-A and B1 showing overall increases of 71.3 % and 114 %, respectively.

The increase in the content of B vitamins during storage could be attributed to the physical-chemical changes in the matrix that continue to release the bound forms of the vitamins. The significantly higher increase in B vitamin content for HP-treated samples can be attributed to higher residual activity of enzymes (Landl et al., 2010; Wibowo et al., 2019). This is in contrast with vitamin C, where the high residual activity of polyphenol-degrading enzymes has a detrimental effect on vitamin C stability, as explained in section 3.1.1. The high residual activity of pectin-degrading enzymes like pectin methylesterase and polygalacturonase can continue degrading the cellular pectin material (Sila et al., 2009), and this can potentially facilitate the release of B vitamins trapped inside it. Additionally, the application of 200–600 MPa under ambient temperature conditions during HP treatment has been reported to commonly result in the activation of enzymes in unfiltered fruit juices and puree (Umair et al., 2022), and the enhanced activity of pectin-degrading enzymes can play a role in significant enhancement of B vitamin content after HP treatment and during storage. The concentrations (mg/L) of B vitamins in the treated nectars during storage are presented in Table B.4 in Appendix B. The calculated % retention (reported in Fig. 3) and the associated uncertainty are presented in Table B.5. in Appendix B.

### 3.5. Color stability during treatment and storage

The color quality parameters for the differently treated strawberry nectar samples after processing and during storage are presented in Table 2. The initial Acceptance Factor (AF) was highest for the TT-treated and lowest for OH-treated samples, but all samples were considered excellent in color (AF > 0.7), demonstrating that all the stabilization techniques are suitable for producing nectars with color that have high consumer acceptance (Murray et al., 2023). After 60 days of cold storage, the AF of the nectars had not significantly changed for PF- and HP-treated samples and a slight but significant increase was seen for TT- and OH-treated samples. The increase in AF during storage can be explained by the fact that AF is a ratio of different color components, and when several components change during storage, such as the changes caused by co-pigmentation reactions that occur during storage (Eiro & Heinonen, 2002), the overall AF can remain constant, or even increase. Based on AF results for the samples, it can be inferred that the color is changing in a way that does not make the nectar less acceptable to consumers. It has previously been shown that the AF of thermally treated strawberry nectars was stable during eight weeks at 4 °C storage (Gössinger, Moritz, et al., 2009), which explains the lack of AF degradation observed in this study.

While AF remained largely constant during storage, total color change  $\Delta E$  did vary between the different treatments. The  $\Delta E_{(0,0)}$ , which corresponds to the color change between the treated and untreated strawberry nectar, was significantly lower for HP-treated samples compared to other technologies, implying that the HP treatment resulted in the least color change due to processing. For TT, PF, and OH treatments,  $\Delta E_{(0,0)}$  was greater than 6, demonstrating that there was a large color difference between the treated and untreated nectar (Lacey et al., 2023). The  $\Delta E_{(0,0)}$  was highest for TT- and OH-treated samples due to their exposure to elevated temperatures, which increase the rate of non-enzymatic browning (Andrés et al., 2016).

The  $\Delta E_{(0,30)}$  and  $\Delta E_{(0,60)}$  is the total color change during storage, between treated nectar on day 0, and after 30 and 60 days of storage at 4 °C, respectively. There was no significant difference between the  $\Delta E_{(0,30)}$

**Table 2**  
Color stability of strawberry nectar during treatment and storage at 4 °C in terms of CIELAB color components and Acceptance Factor (AF).  $\Delta E_{(0,0)}$  corresponds to the color change between the untreated nectar and the treated nectar on day 0.  $\Delta E_{(0,30)}$  corresponds to the total color change between treated samples on day 0 and day 30.  $\Delta E_{(0,60)}$  corresponds to the total color change between treated samples on day 0 and day 60. Lowercase letters in the same column indicate significant differences (n = 3, p < 0.05). TT: conventional thermal treatment, HP: high-pressure processing, OH: ohmic heating, PF: pulse electric field.

Days	L*			a*			b*			C*			h°			AF			Total color change $\Delta E$		
	0	30	60	0	30	60	0	30	60	0	30	60	0	30	60	0	30	60	$\Delta E_{(0,0)}$	$\Delta E_{(0,30)}$	$\Delta E_{(0,60)}$
TT	28.98	30.01	28.20	27.62	17.45	15.05	15.44	32.67	30.38	31.21	32.28	29.69	29.65	0.86	0.92	7.13 ± 0.37c	2.95 ± 1.47a	2.24 ± 0.09a			
	± 0.07c	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±			
	0.99c	21.91	21.87	25.61	0.04b	0.01b	0.03b	0.05b	0.26b	0.16c	0.16c	0.16c	0.88b	0.21b	0.02c	0.01a	0.86	0.86	0.54 ± 0.02a	2.84 ± 0.23a	4.19 ± 0.22c
HP	23.14	± 0.05a	±	23.17	14.94	12.78	11.79	29.65	27.55	25.99	30.26	27.64	26.97	0.85	0.88	±	±	±			
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±			
	0.59a	28.48	27.78	27.90	0.15a	0.15a	0.12b	0.17a	0.69a	0.22a	0.15a	0.44a	0.05a	0.02b	0.82	0.87	0.82	0.82	6.88 ± 0.17bc	2.14 ± 0.14a	3.28 ± 0.78b
OH	29.3	± 0.62c	±	26.28	18.76	16.92	16.44	33.61	32.35	31.00	33.91	31.55	32.01	0.82	0.87	±	±	±			
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±			
	0.04b	27.27	26.11	29.06	0.06c	0.22c	0.12c	0.17c	0.13c	1.07b	0.26c	0.14c	0.77c	0.05a	0.05a	0.04a	0.84	0.88	6.68 ± 0.11b	2.65 ± 0.45a	5.49 ± 0.27d
PF	27.79	±	±	26.04	20.02	17.60	15.76	35.29	33.28	30.44	34.55	31.93	31.18	0.84	0.88	±	±	±			
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±			
	0.15b	0.30b	0.12b	0.04d	0.18b	0.12d	0.35c	0.18b	0.47c	0.25b	0.12d	0.22c	0.12c	0.01b	0.04a	0.02b	0.01b	0.01b			



values of the different treatments, with all treatments in the 'evident' range, implying that the color of all treated nectar changed at a similar rate in the first 4 weeks of storage. In all but the HP-treated nectars the  $\Delta E_{(0,30)}$  was lower than the  $\Delta E_{(0,0)}$ , and so there was a larger color difference between untreated nectar and treated nectar right after treatment (day 0), than treated nectars on day 0 and after 30 days of storage. In the case of HP treatment, however, the magnitude of color change was greater during first 4 weeks of storage for the treated samples than color change that occurred right after processing. As expected, the color of all treated nectars degraded during storage as  $\Delta E_{(0,60)}$  was higher than  $\Delta E_{(0,30)}$  for all treatment but TT-treatment. While the value of  $\Delta E_{(0,30)}$  for TT-treated nectar is higher than  $\Delta E_{(0,60)}$ , the  $\Delta E_{(0,30)}$  has a considerably higher standard deviation. This could be due to a lack of homogeneity in the development of co-pigmentation during storage among the TT-treated samples (Ertan et al., 2020), with some repeats undergoing a larger color change resulting in an overall higher standard deviation. Taking in account the large standard deviation in  $\Delta E_{(0,30)}$  of TT-treated samples, they also seem to follow the apparent trend of degrading color during 60 days of storage. Despite a large deviation in  $\Delta E_{(0,30)}$  there was very little deviation in AF for TT-treated samples, which is suggestive of changes in color components that do not negatively affect consumer perception. After 60 days of storage, TT-treated samples had the lowest  $\Delta E_{(0,60)}$ , significantly lower than all other treatments, and therefore the most color stable treatment over this time period. While the HP-treated samples were reported to have only a slight color change after processing, the samples changed color significantly during storage. The highest color change was reported for PF-treated samples, followed by HP- and then OH-treated samples. The results agree with previous studies which found that TT treatment performed better than HP treatment for color-stable strawberry products (Aaby et al., 2018; Lacey et al., 2023). The significant color change during storage for fruit juices and beverages treated with cold pasteurization technologies like HP and PF treatment has been associated with significant residual enzyme activity i.e. of polyphenol oxidase and peroxidase (Houška et al., 2022; Mannozi et al., 2019). The high color stability of TT- and OH-treated samples can be attributed to a higher degree of enzyme inactivation. Additionally, it can also be correlated with higher retention of AA in TT- and OH-treated samples as reported in the current study. AA has been shown to decrease the rate of the Maillard reaction and can inhibit polyphenol oxidase enzymes, leading to a lower production of browning pigments (Liao et al., 2020; Yu et al., 2021).

AF is a better parameter for assessing color quality and stability since it considers proportional changes in different CIELAB components and can be directly correlated with consumer acceptance (Gössinger, Mayer, et al., 2009). The  $\Delta E$ , on the other hand, reflects on the absolute changes in CIELAB components in a way that is less intuitive in deciding if the change in the overall color quality will affect consumers' behavior towards the product. In such scenarios, sensory tests, i.e. sensory evaluation conducted by panelists using the difference-from-control-test methodology, might be required to make meaningful conclusions. Considering that the AF for all the samples was higher than 0.7 and did not substantially change during storage, it can be concluded that all the preservation technologies successfully preserved the fresh-like color of strawberry nectar after processing and during eight weeks of storage at 4 °C.

#### 4. Conclusion

This study uniquely presented a systematic and comprehensive comparison of the effect of four commercially relevant preservation technologies on all major water-soluble vitamins and color retention in strawberry nectar using pilot-scale equipment. The TT treatment offered the highest retention of vitamin C in strawberry nectar, followed by OH, PF, and HP after treatment and at the end of 8 weeks of storage at 4 °C. Interestingly, the AA content increased significantly after OH, PF, and

TT treatment, with the most profound 15-fold increase observed for TT-treated nectar. The increase in AA content was attributed to its release from the bursting of intact cells under heat stress during TT and OH treatment or electroporation during PF treatment. Puree-derived fruit nectars are an interesting matrix to study as they contain a higher proportion of constituents like fiber, pectin, and other phytochemicals that can alter the behavior of bioactive compounds i.e. vitamins. While the study presented results on how DHA content changed due to processing, they do not necessarily reflect its stability against preservation techniques since the loss of DHA is continuously compensated for by the spontaneous oxidation of AA to DHA. For future research, it is advised to use degradation products of DHA, such as 2,3-diketogluconic acid, as indicators of the extent of its degradation. The B vitamin content in treated strawberry nectar samples remained largely unchanged after processing, and the highest losses were reported for B2 in OH- and TT-treated samples. The B vitamin content increased during eight weeks of storage at 4 °C for most samples. The increase in B vitamin content during storage was attributed to the release of vitamins from their bound forms in the matrix due to residual enzyme activity. While the results presented in the study can be corroborated by objective evidence from the literature, they merit further investigation for a subjective evaluation. Based on the Acceptance Factors (AF), all the preservation technologies investigated in the current study resulted in similar color quality after treatment and during storage at 4 °C for eight weeks. Conducting further trials at an elevated storage temperature, i.e., 10 °C or 20 °C, may offer further insight into color stability in differently treated strawberry nectar samples in future studies.

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#### CRediT authorship contribution statement

**Hassan Zia:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Helen Murray:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Mikko Hofsommer:** Writing – review & editing, Supervision, Resources. **Andrés Moreno Barreto:** Writing – review & editing, Methodology. **Darío Pavon-vargas:** Writing – review & editing, Methodology. **Alema Puzovic:** Writing – review & editing, Methodology. **Astrid Gędas:** Writing – review & editing, Methodology. **Sebastian Rincon:** Writing – review & editing, Methodology. **Manfred Gössinger:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ana Slatnar:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Data availability

Data will be made available on request.

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