

Research Article

Copigmentation with Sinapic Acid Improves the Stability of Anthocyanins in High-Pressure-Processed Strawberry Purees

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This study investigated the impact of copigmentation with sinapic acid on the stability of anthocyanins in strawberry purees of three commercial cultivars (Camarosa, Rubygem, and Festival) after high-pressure processing (HPP; 600 MPa/5 min) and thermal processing (TP; 88°C/2 min) and during three months of refrigerated storage. Copigmentation did not have a significant effect on the stability of anthocyanins during processing with 14% to 30% degradation observed with no significant difference among cultivars or the processing technique. On the contrary, copigmentation significantly ($p < 0.05$) improved the stability of anthocyanins in HPP samples during storage, most probably via the formation of intramolecular complexes which improve the resistance of anthocyanins to degradation. The anthocyanin contents of the copigmented HPP Camarosa, Rubygem, and Festival samples were, respectively, 42%, 40%, and 33% higher than their noncopigmented counterparts at the end of the three-month storage. Copigmentation also improved the retention of the total antioxidant capacity of the HPP-processed strawberry samples. The TPC of the copigmented HPP Camarosa, Rubygem, and Festival samples was, respectively, 66%, 65%, and 85% higher than that of the non-copigmented samples after three months of storage, whereas the respective ORAC values were 36.5%, 59.3%, and 35.3% higher. In contrast, copigmentation did not improve the stability of anthocyanins in TP samples, although significant ($p < 0.05$) improvement in antioxidant capacity was also observed in TP samples due to the antioxidant nature of the copigment.

1. Introduction

Anthocyanins, water-soluble plant pigments and a major subclass of polyphenols, are of significant interest due to their potential role in human health. There is growing epidemiological and also experimental evidence that anthocyanins and anthocyanin-rich foods may have beneficial (preventative) effects on cognition, vision, blood pressure, and cardiovascular disease risk factors [1]. Strawberry (*Fragaria × ananassa*) is one of the most popular and commonly consumed fruits worldwide. The fresh fruit and processed products can be rich in bioactive phytochemicals such as anthocyanins and other polyphenols, depending on the cultivar, pre- and postharvest treatment, and the

processing technology used. Although more than 25 anthocyanin compounds have been identified in different strawberry cultivars to date, the most common are pelargonidin-3-glucoside as the main pigment (77–95% of total anthocyanins) followed by pelargonidin-3-rutinoside and cyanidin-3-glucoside [2–4]. These natural pigments are not only interesting because of their reported potential health benefits but also because of their characteristic colour attributes (responsible for the red colour of strawberries). However, the colour of most strawberries deteriorates significantly during processing and storage due to the degradation of anthocyanins. Intrinsic factors related to the food matrix such as pH, enzyme activity, the presence of metal ions, and the structure of anthocyanins as well as factors

such as temperature and the availability of oxygen during processing and storage influence the rate and extent of degradation [5, 6].

Our previous studies showed limited effects of high-pressure processing on anthocyanins and other polyphenols in strawberry halves [7] and purees [5]. Nevertheless, a substantial loss of anthocyanins occurred during refrigerated storage of the products which was attributed to the residual activities of endogenous enzymes such as β -glucosidase, polyphenol oxidase, and peroxidase [5, 7, 8]. Previous studies have shown that copigmentation with phenolic acids and phenolic-rich plant extracts improve the stability of anthocyanins via intermolecular and intramolecular complexations [6]. This has been demonstrated for sweetened strawberry juice drinks [9] and other products [10, 11]. Rein and Heinenon [9] compared the efficacy of ferulic acid, sinapic acid, and rosemary extract for enhancing and stabilising anthocyanins in sweetened strawberry juice. Sinapic acid provided the highest colour enhancement and stability during 103 days of storage of the juice at room temperature [9]. The aim of this study was to evaluate the feasibility of copigmentation with sinapic acid to improve the storage stability of anthocyanins in high-pressure- and thermally processed strawberry purees.

2. Materials and Methods

2.1. Chemicals. Pelargonidin-3-glucoside (pel-3-glc) and cyanidin-3-glucoside (cya-3-glc) were purchased from ChromaDex (Irvine, CA, USA). All the other chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) or Merck (Kilsyth, VIC, Australia) and were of analytical or HPLC grade. Milli-Q water was used throughout unless otherwise stated.

2.2. Strawberry Puree Samples Preparation. Fresh ripe strawberries from three commercial cultivars (Camarosa, Festival, and Rubygem) were obtained from a local Queensland (Australia) grower and individually quick frozen for transport to Melbourne where processing trials were conducted. The strawberries were stored at -20°C until processing. Prior to processing, the strawberry samples were thawed by keeping them overnight at 4°C . After thawing, the calyx and peduncles of the strawberries were removed, and the strawberries were pureed using a laboratory scale homogenizer (Model Ruby 2000, Nutrifaster Australia, Wetherill Park, NSW, Australia). The strawberry purees from the three cultivars were divided into two lots. In one lot, sinapic acid was added at 5 to 1 molar ratio with the anthocyanins present in the strawberry puree samples. Samples from the two lots (200 g) were put in oxygen barrier flexible plastic bags (NYLON/EVOH/PE structure, $70\ \mu\text{m}$, OTR $b8\ \text{mL}/\text{m}^2/\text{day}/\text{atm}$ at $23^{\circ}\text{C}/75\% \text{RH}$ and 1 atm, Holmes Packaging, Fawkner, VIC, Australia) and were sealed for immediate processing.

2.3. Thermal and High-Pressure Processing. The strawberry puree samples from the three cultivars were processed as described in Terefe et al. [5]. Thermal pasteurization ($88^{\circ}\text{C}/2\ \text{min}$) of the samples was conducted for 2 min at 88°C

in a thermostated water bath. The 2 min treatment time was after temperature equilibration at the slowest heating point in the samples. High-pressure processing was conducted for 5 min at 600 MPa and 20°C in a 35 L high-pressure vessel (Flow Pressure System QUINTUS® Food Press Type 35L-600 sterilization machine, Avure Technologies, Lincoln, NE, USA). The samples and the processing water were maintained at 4°C prior to processing in order to achieve the target temperature of 20°C after compression. A compression rate of 4.2 MPa/s and a decompression rate of 40 MPa/s were used in the experiments. All the experiments were conducted three times. Randomly selected samples from each replicate were immediately frozen for subsequent phytochemical analyses after freeze-drying, blending, and extraction steps. The rest were kept for three months at 4°C for storage stability studies after which they were frozen prior to analysis.

2.4. Analysis of Anthocyanins. The extraction procedure was carried out as described by Fredericks et al. [4] and Netzel et al. [12] using a mixture of MeOH/ H_2O /formic acid (80/19/1; v/v/v) as the extraction solution. Strawberry anthocyanins were analysed on a Aqua Luna C18 (2) reverse-phase column using a Waters 600E HPLC system as described previously [4, 13]. Anthocyanins were quantified using external cya-3-glc and pel-3-glc calibration curves and were calculated as μmol per 100 g sample. Pelargonidin-3-rutinoside (pel-3-rut) was quantified via the pel-3-glc calibration curve. Identification of anthocyanin compounds was carried on a Thermo Finnigan Quantum triple-stage quadrupole mass spectrometer [4].

2.5. Analysis of Antioxidant Capacity. The total phenolic content (TPC) as a typical electron transfer (ET) assay measuring (poly)phenolics and other reducing compounds was conducted as described previously [4, 14]. TPC was expressed as mg gallic acid equivalents (GAE) per 100 g sample. The oxygen radical absorbance capacity (ORAC) assay as a typical hydrogen atom transfer (HAT) assay was conducted as described by Fredericks et al. [4] and Huang et al. [15]. ORAC results were expressed as mmol of Trolox equivalents (TE) per 100 g sample.

2.6. Statistical Analysis. One-way analysis of variance (ANOVA) was used to determine whether copigmentation has a statistically significant effect on the stability of anthocyanins and other (poly)phenolics/antioxidants (as antioxidant capacity) following processing and during storage. The significance of differences between treatment means was evaluated using the Tukey multiple range test at the 0.05 level of significance. All data analyses were conducted using Design expert software, version 7.1.3 (Stat-Ease, Inc., Minneapolis, MN, USA) and Origin pro version 8.5 (OriginLab Corp., Northampton, MA, USA).

3. Results and Discussion

3.1. Anthocyanins and Antioxidant Capacity of Unprocessed Strawberry Fruit. The total anthocyanin contents of the

fresh/unprocessed commercial strawberry cultivars Camarosa, Rubygem, and Festival ranged from 64.2 to 91.9 $\mu\text{mol}/100\text{ g}$ (equivalent to 27.8–39.7 mg/100 g) (Table 1), and it fell within the range of 8.5–105 mg/100 g anthocyanins described in previous studies for commercial cultivars and specific breeding lines [3, 4, 16–23]. Furthermore, the anthocyanin profile and relative proportions of the various anthocyanins (pel-3-glc, pel-3-rut, and cya-3-glc) were in agreement with the results reported by others with pel-3-glc as the predominant anthocyanin in all cultivars [2–4, 24]. The results for the total phenolics and ORAC were found to be within the literature range of 153.1–420 mg GAE/100 g [4, 17, 25, 26] and 3.27–6.77 mmol TE/100 g, respectively [4].

3.2. The Effects of Copigmentation on the Stability of Anthocyanins following Processing and during Refrigerated Storage.

The total anthocyanin content of the strawberry puree samples following HPP and TP significantly decreased ($p < 0.05$) by 14 to 30% except in the case of Camarosa samples where the decrease after thermal processing was not statistically significant ($p > 0.05$) (Table 1). Copigmentation with sinapic acid did not have a significant ($p > 0.05$) effect on anthocyanin retention following both TP and HPP in any of the samples with no significant difference between copigmented and non-copigmented samples of the same cultivar after processing. In contrast, copigmentation of grape juice with rosemary and thyme extract had a substantial effect on anthocyanin retention in the juice following a 15 min treatment at 400 MPa and 550 MPa [11]. In neat juice, ~70 and 44% degradation of anthocyanin, respectively, was observed after processing at 400 MPa and 500 MPa, respectively, which was much higher than what we observed in our strawberry samples after high-pressure processing. This could be attributed to the observed 3 and 2.5 times increase in the activity of polyphenol oxidase in the grape juice after processing at 400 MPa and 550 MPa. In the samples with added rosemary extract, the retention of anthocyanins was almost 100% after processing at both 400 and 550 MPa, whereas 15% and 9% loss were observed after processing at 400 MPa and 550 MPa, respectively, in samples with added thyme extract [11]. It has to be noted that both extracts contain anthocyanins as well as various polyphenols, which may have been resulted in both intermolecular and intramolecular complex formation, which may have rendered the anthocyanin resistant to enzymatic degradation during high-pressure processing. In addition, the anthocyanin-copigment molar ratio was 1 to 100 [11] compared to the 1 to 5 ratio in this study.

During storage, substantial degradation of anthocyanins occurred in both HPP and TP samples ($p < 0.05$) (compare Table 1 and Figure 1). Based on the anthocyanin content at day zero after processing, 60.6% to 68.7% degradation of anthocyanins occurred during three months of refrigerated storage of copigmented HPP samples compared to 71.4% to 76% in the non-copigmented HPP samples. With regard to TP samples, the degradation during storage was 62.3% to 67.6% in the copigmented samples compared to 64.6% to 67.5% in non-copigmented samples. Analysis of variance

TABLE 1: Antioxidant capacity and total anthocyanin contents of fresh/unprocessed commercial strawberry samples and processed purees with and without added sinapic acid.

Cultivars/treatment	TPC	Total anthocyanins	ORAC
Unprocessed Camarosa	265.2 \pm 9.2 ^a	83.2 \pm 0.81 ^a	4.4 \pm 0.05 ^a
Unprocessed Rubygem	236.2 \pm 6.1 ^b	64.2 \pm 0.45 ^b	4.2 \pm 0.20 ^{ab}
Unprocessed Festival	239.9 \pm 6.3 ^b	91.9 \pm 2.22 ^c	3.7 \pm 0.16 ^b
Camarosa TP-NP	209.9 \pm 5.1 ^c	64.7 \pm 2.4 ^{ad}	2.9 \pm 0.07 ^c
Camarosa HPP-NP	216.4 \pm 1.5 ^c	61.7 \pm 2.92 ^d	2.9 \pm 0.08 ^c
Camarosa TP-CP	289.0 \pm 2.1 ^{ad}	71.7 \pm 1.71 ^{ad}	5.5 \pm 0.02 ^d
Camarosa HPP-CP	295.9 \pm 9.8 ^d	63.8 \pm 8.31 ^d	5.1 \pm 0.38 ^{ad}
Rubygem TP-NP	179.8 \pm 6.9 ^e	48.3 \pm 3.85 ^e	2.6 \pm 0.03 ^e
Rubygem HPP-NP	183.3 \pm 1.2 ^e	46.5 \pm 1.29 ^e	2.5 \pm 0.15 ^e
Rubygem TP-CP	251.1 \pm 3.2 ^b	53.5 \pm 0.98 ^e	3.5 \pm 0.2 ^f
Rubygem HPP-CP	247.0 \pm 1.6 ^b	44.7 \pm 0.8 ^e	2.8 \pm 0.1 ^{ef}
Festival TP-NP	206.1 \pm 3.5 ^b	67.6 \pm 0.24 ^f	2.9 \pm 0.09 ^{bg}
Festival HPP-NP	218.1 \pm 13.1 ^b	73.5 \pm 6.92 ^f	2.8 \pm 0.34 ^g
Festival TP-CP	292.4 \pm 11.6 ^f	65.9 \pm 1.7 ^f	3.5 \pm 0.12 ^{bg}
Festival HPP-CP	305.7 \pm 2.9 ^f	75.1 \pm 3.82 ^{cf}	3.7 \pm 0.32 ^{bg}

Data: means \pm SD ($n = 3$). Results are expressed in mg GAE/100 g FW (TPC), $\mu\text{mol}/100\text{ g}$ FW (anthocyanins), and mmol TE/100 g FW (ORAC). FW: fresh weight; anthocyanins: sum of cya-3-glc, pel-3-glc, and pel-3-rut (expressed as pel-3-glc); TP-NP: non-copigmented thermally processed; TP-CP: copigmented thermally processed; HPP-NP: non-copigmented high-pressure processed; HPP-CP: copigmented high pressure processed; TPC: total phenolic content. Different letters within a group in a column indicate a significant difference ($p < 0.05$), the groups being unprocessed samples, processed Camarosa samples together with unprocessed Camarosa, processed Rubygem samples with unprocessed Rubygem, and processed Festival samples with unprocessed Festival.

showed that, overall copigmentation had a significant ($p < 0.05$) effect on the stability of anthocyanins during storage. Comparing the individual means, copigmentation significantly ($p < 0.05$) improved the stability of anthocyanins in the HPP-treated samples from all cultivars. A 42%, 40%, and 33% higher amount of anthocyanins was observed in copigmented HPP Camarosa, Rubygem, and Festival samples, respectively, after storage compared to the non-copigmented HPP samples (Figure 1). On the contrary, copigmentation did not have a significant effect on the stability of anthocyanins in TP samples during storage except for Camarosa samples where copigmented samples had on average a 28.3% higher anthocyanin content compared to samples without an added copigment after three months of refrigerated storage. The copigmented Camarosa samples had a higher average anthocyanin content after thermal processing compared to the samples without added copigment. This may have contributed to the observed higher anthocyanin content of the thermally processed copigmented Camarosa samples after storage. Regardless of the processing approach, a slightly better retention of anthocyanins was observed during storage of copigmented Camarosa samples compared to Rubygem and Festival, which could be due to the lower polyphenol oxidase activity of these samples [5].

It is well known that anthocyanins form intramolecular complexes with hydroxycinnamic acids such as sinapic acid

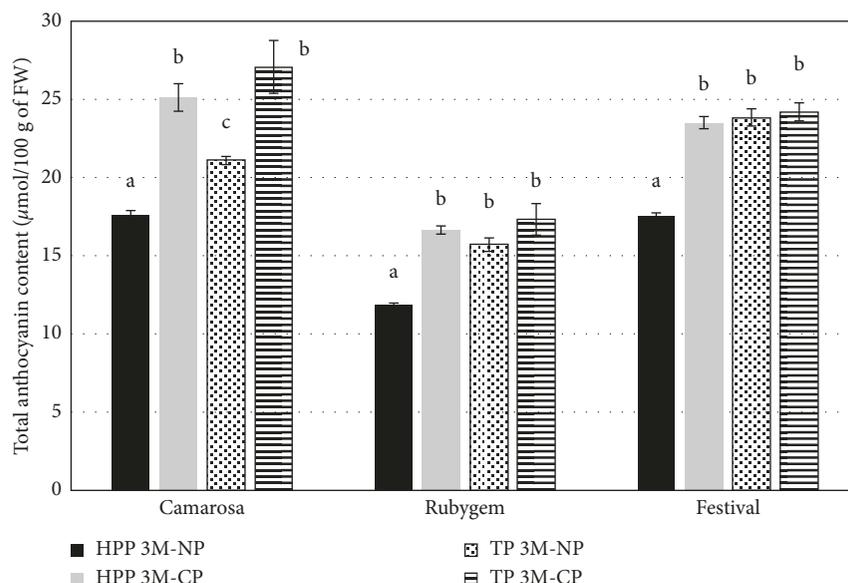


FIGURE 1: The effects of copigmentation on anthocyanin content of high-pressure- (HPP) and thermally processed (TP) strawberry puree samples after three months of refrigerated (4°C) storage. CP-3M: copigmented samples and NP-3M: non-copigmented samples stored for three months at 4°C. Different letters within a group designate a significant difference ($p < 0.05$).

[6]. A study by Rein and Heinonen [9] showed that sinapic acid is an effective copigment for strawberry anthocyanins and forms intramolecular copigmentation molecules with strawberry anthocyanins [9]. The observed better stability of anthocyanins in copigmented HPP-processed juice could be due to the formation of such complexes, which may be resistant to degradation by the residual activities of endogenous enzymes. Nevertheless, degradation of anthocyanins was not completely inhibited during storage even in the HPP-treated copigmented purees. This could be due to incomplete conversion of the anthocyanins into intramolecular complexes with sinapic acid under the studied condition and possible disruption of these complexes by high pressure prior to storage. In the study by Del Pozo-Insfran et al. [11] discussed above, copigmentation of grape juice by thyme and rosemary extracts did not reduce the rate of degradation of anthocyanins in high pressure processed (400 MPa, 550 MPa) juices during storage. However, a higher content of anthocyanins was observed in the copigmented juices most likely due to a better retention after processing as well as the additional amount of anthocyanins from the extracts that were used for copigmentation. Interestingly, copigmentation did not improve the storage stability of anthocyanins in thermally treated strawberry puree samples in this study. This could be due to the susceptibility of the copigment complexes to thermal degradation. A study by Malien-Aubert et al. [27] suggests that intramolecular complexes formed by non-acylated anthocyanins like strawberry anthocyanins have relatively low thermal stability.

The degradation mechanism of anthocyanins during high-pressure and thermal processing differ. During high-pressure processing, degradation is likely due to the synergistic activities of endogenous glycosidases and oxidases or through coupled oxidation mechanisms that involve oxidases and other compounds such as polyphenols and

ascorbic acid [5, 11] naturally present in strawberries. The degradation of anthocyanins during thermal processing on the other hand involves nonenzymatic oxidation and cleavage of covalent bonds, which continue during storage via intermediates formed during processing [28]. The differences in the anthocyanin degradation mechanism during high-pressure and thermal processing may explain the observed difference in the stabilising effect of sinapic acid in thermally processed and high-pressure-processed strawberry puree samples.

3.3. The Effects of Copigmentation on the Antioxidant Capacity following Processing and during Refrigerated Storage. The total polyphenol content (TPC) significantly ($p < 0.05$) increased after processing of copigmented strawberry samples (Table 1), since the added copigment itself is a phenolic acid with antioxidant activity. The total phenolic content after processing was 5% to 27% higher than the fresh samples in the copigmented sample batch (Table 1). The highest increase in TPC occurred in copigmented Festival samples with about 27% increase after HPP and 22% increase after thermal processing, which could be at least partly due to the higher amount of sinapic acid added in proportion to the higher anthocyanin content of Festival samples. On the contrary, a significant reduction in TPC of 9% to 23% was observed in the non-copigmented samples after processing. The lowest reduction after processing of the non-copigmented samples was also observed in Festival samples, with 9% reduction after HPP and 14% reduction after thermal pasteurization. This could indicate that the observed higher increase in TPC in the copigmented Festival samples is the cumulative effect of the higher quantity of sinapic acid added and lesser degradation during processing compared to the other cultivars.

During three months of refrigerated storage (4°C), 21% to 31% decrease in TPC was observed in copigmented HPP samples compared to samples just after processing. A slight but significantly higher decrease in TPC of 27% to 38.7% was observed in the non-copigmented HPP samples, reflecting the observed lower storage stability of anthocyanins in the non-copigmented HPP samples (Compare Table 1 with Figure 2). The highest decrease of 38.7% was observed in the non-copigmented Festival samples after HPP. On the contrary, a slightly lower reduction in TPC during storage was observed in the thermally processed samples compared to HPP samples. The average decrease in TP samples ranged from 15% to 24% in the copigmented samples and 17% to 23% decrease in the non-copigmented samples. There was no significant difference between the thermally processed copigmented and non-copigmented samples in the percentage TPC reduction during storage. This is in line with the result observed for total anthocyanins. The lowest decrease in TPC of thermally processed samples during storage was observed for Festival samples, which is opposite to the trend observed with HPP samples perhaps reflecting the differences in the degradation mechanisms of antioxidant compounds in thermally and HPP-processed products. The TPC of copigmented HPP samples after 3-month refrigerated storage was 66%, 65%, and 85% higher than the TPC of non-copigmented Camarosa, Rubygem, and Festival, respectively, compared to 36.7%, 34.7%, and 40.1%, respectively, just after processing. The TPC of the heat-treated samples on the contrary was 52%, 42%, and 55% higher for Camarosa, Rubygem, and Festival, respectively, after storage compared to 37.6%, 39.6%, and 41.8% just after processing. The higher relative difference after storage especially for the HPP samples reflects the protective effect of copigmentation on anthocyanins during storage of HPP-processed products. Overall, it is obvious that copigmentation has an advantage in augmenting and stabilising the antioxidant capacity of products with antioxidant compounds susceptible to degradation during extended storage.

As in the case of TPC, addition of sinapic acid had a significant ($p < 0.05$) effect on the ORAC antioxidant capacity of both high-pressure- and thermally processed strawberry puree samples (Table 1). However, unlike TPC, a significant ($p < 0.05$) increase in ORAC after processing was observed only in Camarosa samples, whereas on average 16% and 25% higher ORAC values were observed for the copigmented high pressure and thermally processed samples, respectively, compared to the fresh sample. In the non-copigmented Camarosa samples, 34% and 35% reductions in ORAC values, respectively, were observed following thermal and high-pressure processing. Similarly, 33% and 40% reductions, respectively, in ORAC values were observed in copigmented and non-copigmented Rubygem strawberry purees after high-pressure processing, whereas 33% and 38% reductions were observed after thermal processing of the same samples. In the case of Festival, no significant change in ORAC was observed after HPP and thermal processing of copigmented samples. In the case of the non-copigmented Festival samples, 26% and 23% reduction in ORAC was observed after HPP and TP. The highest reduction in ORAC

antioxidant capacity after processing of ~40% was observed in Rubygem samples after HPP of non-copigmented samples. Overall, copigmentation improved the retention of ORAC antioxidant capacity of Rubygem samples after processing (Table 1), although the difference was not statistically significant in the case of HPP Rubygem samples ($p > 0.05$).

In all cases, the effect of copigmentation on ORAC was not as substantial as in the case of TPC. It seems that the added sinapic acid and the complexes that are formed do not contribute substantially to the ORAC antioxidant capacity of the strawberry samples. In contrast, Del Pozo-Insfran et al. [11] observed about 60% increase in ORAC antioxidant capacity after HPP processing (400 MPa, 550 MPa, 15 min) of Muscadine grape juice with added rosemary and thyme extracts as copigments.

The effect of refrigerated storage on ORAC antioxidant capacity did not follow a clear trend. It increased or stayed constant except in copigmented Camarosa samples where an increase in ORAC was observed after thermal and high-pressure processing (Table 1 and Figure 3). The ORAC values decreased by 27% and 19%, respectively, in copigmented thermally processed and high-pressure-processed Camarosa samples during storage. On the contrary, the ORAC values of the non-copigmented HPP and TP Camarosa samples increased by ~9% and 32%, respectively, after three months of storage. Similarly, a significant increase of 9%, 40%, and 25% in ORAC was observed in TP copigmented, HPP copigmented, and TP non-copigmented Rubygem samples, respectively, during storage. There was also ~9% increase in ORAC values of copigmented thermally processed Festival samples. No significant change was observed in the other samples (Figure 3).

The observed increase in ORAC antioxidant capacity of some of the samples during storage could be attributed to the formation of degradation products with antioxidant capacity. It is known that some degradation products of polyphenolic compounds possess antioxidant capacity [28]. Regardless of the differences in the evolution of ORAC during processing and storage of strawberry samples, it is obvious that copigmentation resulted in a significantly higher ORAC values after storage of all samples (Figure 3). The ORAC values were 36.5%, 59.3%, and 35.3% higher in copigmented Camarosa, Rubygem, and Festival samples, respectively, after three months of storage of the HPP samples compared to the non-copigmented samples. A slightly less effect was observed in the TP samples where the ORAC values were 13.2%, 17.3%, and 31.1% higher in copigmented Camarosa, Rubygem, and Festival samples, respectively, after three months of storage compared to the non-copigmented samples. The result clearly reflects the protective and stabilising effect of copigmentation on anthocyanins and perhaps other antioxidants during storage of processed strawberry samples.

The observed different trend between TPC and ORAC could be due to the difference in the chemical principles between the two measures of total antioxidant capacity: the former being an electron transfer assay, while the latter is a hydrogen transfer assay [15]. This sort of difference in

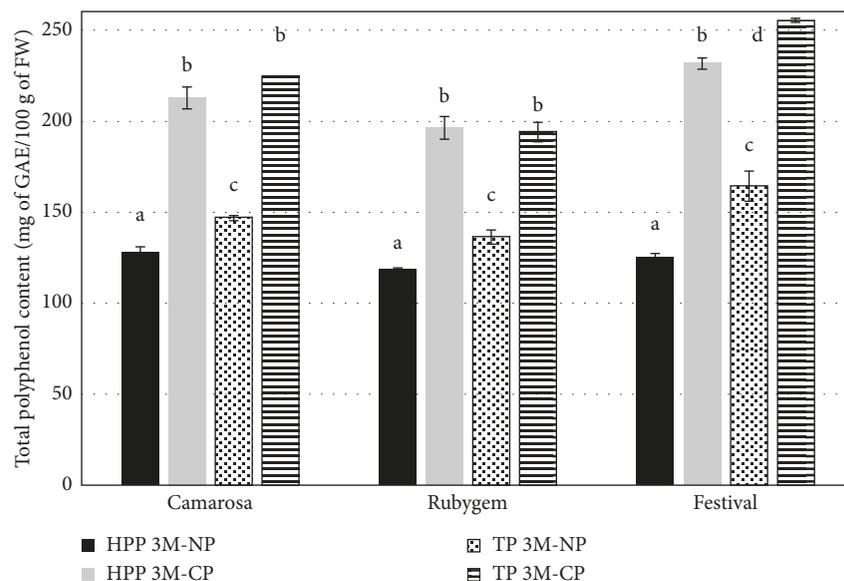


FIGURE 2: The effects of copigmentation on total phenolic content following three months of refrigerated (4°C) storage of HPP and TP strawberry puree samples. CP-3M: copigmented samples and NP-3M: non-copigmented samples stored for three months at 4°C. Different letters within a group designate a significant difference ($p < 0.05$).

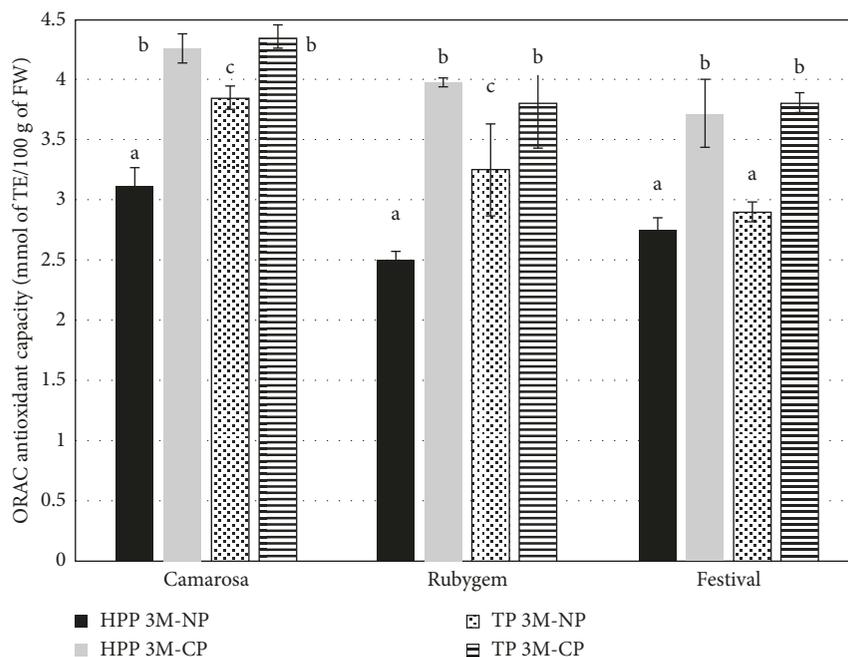


FIGURE 3: The effects of copigmentation on ORAC antioxidant capacity following three months of refrigerated (4°C) storage of HPP and TP strawberry puree samples. CP-3M: copigmented samples and NP-3M: non-copigmented samples stored for three months at 4°C. Different letters within a group designate a significant difference ($p < 0.05$).

observed trends between TPC and ORAC has been reported in several studies [5, 29].

4. Conclusions

The present study showed that copigmentation with sinapic acid significantly improves the storage stability of anthocyanins in high-pressure-processed strawberry puree samples. In samples copigmented with sinapic acid at 5 to

1 copigment-anthocyanin molar proportion, the retention of anthocyanins after three months of storage was 42%, 40%, and 33% higher in Camarosa, Rubygem, and Festival compared to the non-copigmented samples. This can most likely be attributed to the formation of relatively stable intramolecular complexes with sinapic acid, which have shown better resistance to degradation during storage by oxidation and other mechanisms. Interestingly, copigmentation did not have a significant impact on the storage

stability of anthocyanins in thermally processed strawberry samples, perhaps due to the susceptibility of the intramolecular complexes to heat degradation. Copigmentation also improved the retention of antioxidant capacity of both TP and HPP samples after processing, which could be attributed to the added sinapic acid which is an antioxidant and the stabilisation of anthocyanins. The TPC of the copigmented samples after three months of storage were 42% to 55% higher in TP samples and 65% to 85% higher in HPP samples compared to their non-copigmented counterparts. The higher retention in the HPP samples is a reflection of the anthocyanin stabilising effect of copigmentation in high-pressure processed strawberries. Nevertheless, a substantial loss of anthocyanins (60.6% to 68.7%) occurred during storage of copigmented HPP samples. Thus, further improvement is required via optimisation of the copigment to anthocyanin ratio and inclusion of a preprocessing step to allow sufficient complex formation prior to high-pressure processing.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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