

*Effects of storage on fruit juice quality:
Antioxidants, polyphenols and vitamin C*

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EXECUTIVE SUMMARY

1. Two juice mixtures were analysed for total phenol content, anthocyanin content, antioxidant capacity, insoluble material and vitamin C content. The stability of these characteristics was measured in one juice mixture (1HPP) over 6 weeks at two storage temperatures (4°C and 18°C).
2. The two juices (1 and 2) had at least as much total phenol content as the commercial comparator juice (purple grape juice). This was before the contribution of phenols still attached to the insoluble berry puree could be taken into account. Pasteurised juices had higher levels than high pressure treated juices. However, both juices 1 and 2 had higher antioxidant capacity than PGJ as measured by FRAP.
3. The total anthocyanin content of both juices was significantly higher than PGJ.
4. The two juices had notable amounts of insoluble material (~ 1mg dry weight per 100 mL) which reflects dietary fibre content. The high pressure treated juices had higher contents than the pasteurised forms.
5. Juice mixture 1 had a higher vitamin C content than juice mixture 2 (at ~ 20 mg /100 mL), which equates to 50 % RDA based on the Food Standards Agency figures. High pressure treated juices retained more vitamin C than pasteurised juices.
6. The total phenol content of juice 1HPP dropped between one and two weeks of storage at both 4 and 18°C but did not change markedly over the following six weeks. The antioxidant capacity (FRAP value) dropped to approx 85 % of original after one week of storage but then did not change greatly.

7. The total anthocyanin content of the juice dropped in a linear fashion upon storage at both 4 and 18°C. However, the rate of decline was significantly faster at 18°C. Therefore, after three weeks the anthocyanin content at 18°C was < 60 % of the original value whereas at 5°C this value was 90 %.
8. Vitamin C content declined notably after one week storage at 18°C (75 % original value) but did not decline further over six weeks. Vitamin C content at 4°C was more stable (> 85 % original value at six weeks)
9. LC-MS analysis of the polyphenol profile confirmed that the juice mixture 1 contained components characteristic of strawberry, black currant, raspberry, red currant and aronia (and roughly in that order of abundance). The wide diversity of polyphenol components could be a unique aspect of this juice.
10. LC-MS analysis also showed that the drop in total anthocyanin content noted upon storage at 18°C was caused by a consistent reduction in all anthocyanin types.

JUICE MIXTURE RESULTS

Juice mixture 2 has significantly higher phenol content than juice mixture 1 (Fig. 1).

The two juice mixtures have at least as much phenol content as the purple grape juice.

Juice 1HPP did not have significantly higher phenol content than PGJ (p set at 0.1).

However, this is before the contribution of phenols still attached to the insoluble puree is taken into account. These insoluble phenols could be mobilised during digestion in the gut and contribute to health beneficial effects (Saura-Calixto, 2011).

The pasteurised juices have higher levels than the high pressure treated juices probably due to more effective extraction of phenols from the purees into the soluble phase which was measured.

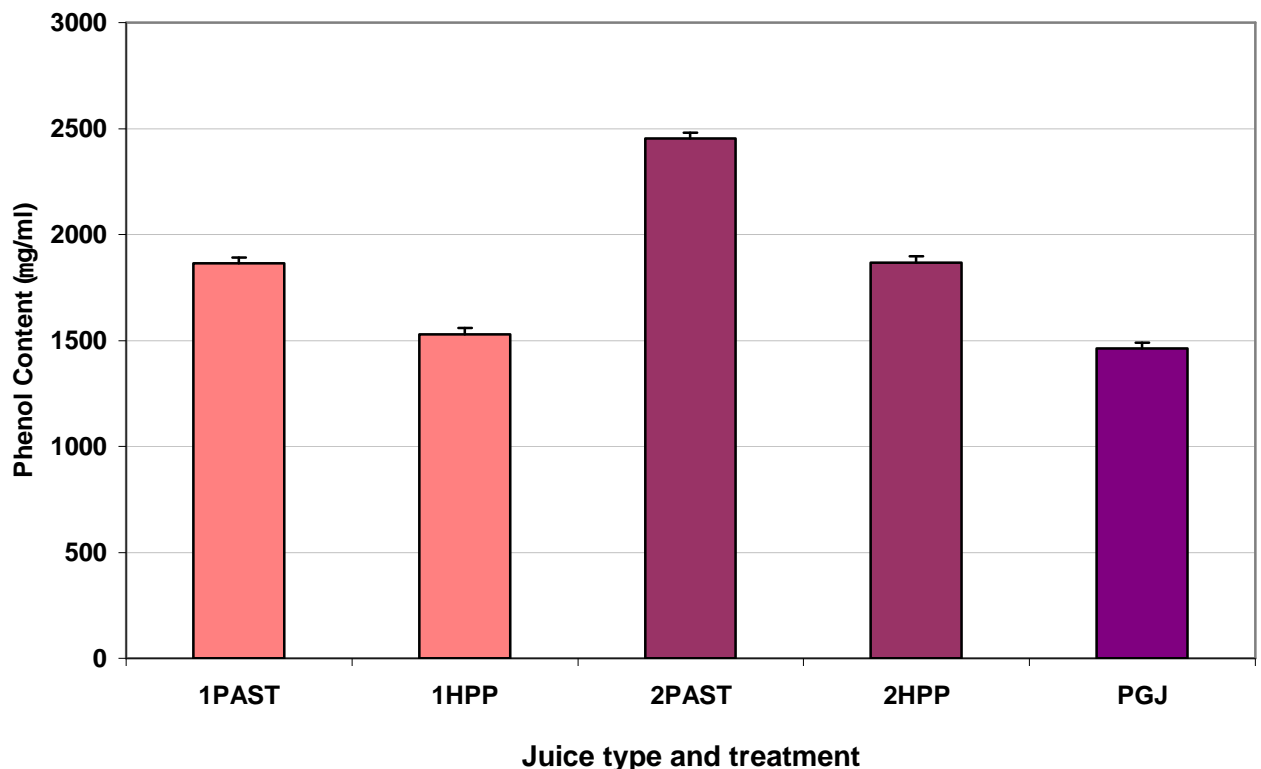


Fig. 1 Effect of juice type and treatment on total phenol content – PAST = pasteurised, HPP = high pressure treated.

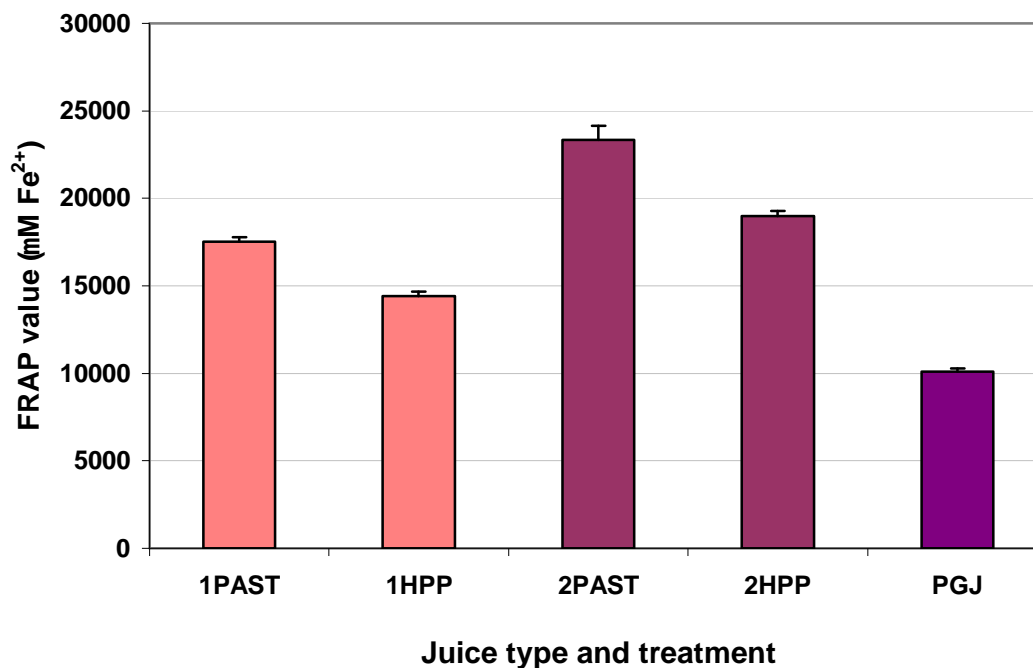


Fig. 2 Effect of juice type and treatment on total antioxidant capacity (FRAP)

The FRAP values (measurement of antioxidant capacity or AOC) mirror the total phenol values apart from Purple Grape Juice which seems to have a lower relative AOC. This is surprising as Mullen et al (2007) rated this purple grape juice product very highly on FRAP levels but they did not look at mixtures similar in composition to juice 1. The diversity of polyphenol content from the different source juices, as revealed by LC-MS (Annex), may cause this high AOC.

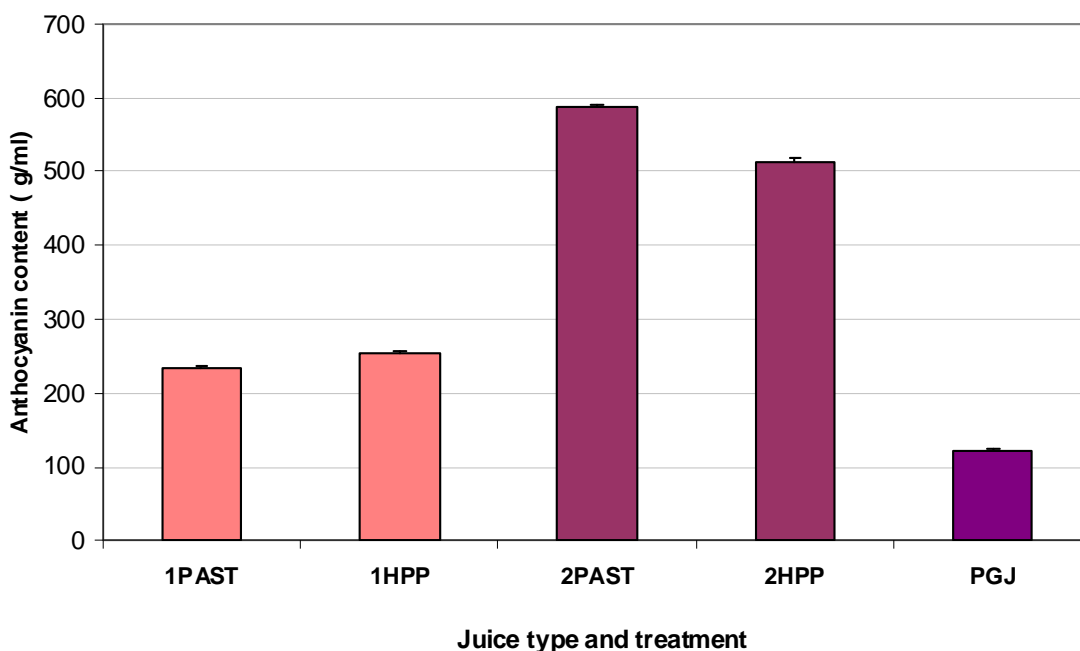


Fig. 3 Effect of juice type and treatment on total anthocyanin content

The high pressure treated juice 1 had higher anthocyanin content than the pasteurised juice but pasteurised juice 2 had higher anthocyanin content than the high pressure treated juice 2.

However, the ratio of the total anthocyanin content was higher in the high pressure treated juices the pasteurised juices (anthocyanin/phenol ratios of 0.125 vs. 0.165; 1PAST vs. 1HPP and 0.239 vs. 0.274 for 2PAST and 2HPP). This could be due to preferential extraction of anthocyanins by the high pressure treatment or, perhaps more likely, to increased degradation of anthocyanins by pasteurization.

Juice mixture 2 yielded more insoluble dry material content than juice mixture 1 (Fig. 4). This is a reliable but mainly comparative estimate of dietary fibre content. *Any final juice product would need to have an accredited dietary fibre assay to allow any claim on dietary fibre content.* The high pressure treated juices had higher dry matter content than the pasteurised juices and this statistically significant for juice mixture 2 (p value < 0.005).

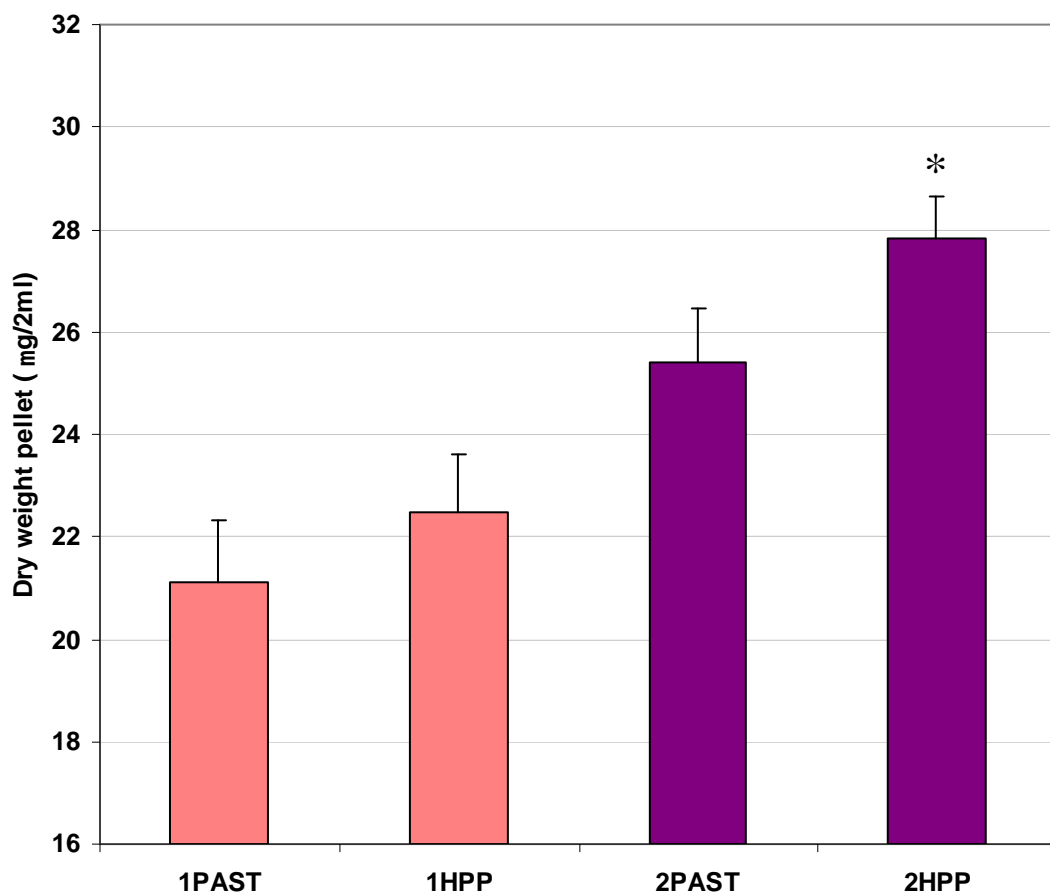


Fig. 4 Effect of juice type and treatment on dry matter content

Juice mixture 1 had higher vitamin C content than juice 2 (Fig. 5) and high pressure treatment appeared to yield higher vitamin C content. The difference was significant for juice 1 ($p < 0.01$) but juice 2 showed the same trend. This level of vitamin C (~ 20 mg/100 ml juice) equates to 50 % of recommended daily intake in the UK as quoted on the NHS website

(<http://www.nhs.uk/conditions/diet/pages/vitaminsandminerals.aspx>).

However, using this data to make a claim about high or a good source of vitamin C would depend on the formulation of the final juice mixture and may need to be checked again.

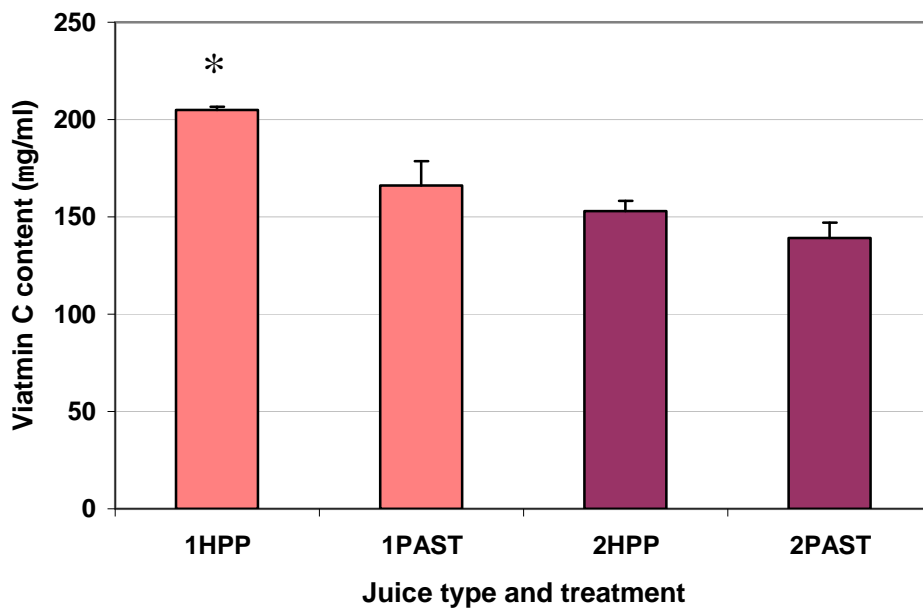


Fig. 5 Effect of juice type and treatment on vitamin C content

EFFECTS OF STORAGE

Appearance and sensory qualities

The effect of long term storage of juice 1HPP at 5 and 18°C was studied. There was little obvious effect of storage at either temperature on colour or juice appearance and photographs did not show any obvious differences. However, it seemed that juice stored at 18°C was less viscous from week 3 onwards but this was only noticed through pipetting the juice. However this could change important mouth-feel and residency characteristics. This apparent change could be checked by assessing dry matter content or directly measuring viscosity in these samples.

A subtle change in odour was noted after three weeks. The juice stored at room temperature (18°C) was less sweet smelling than the cold-stored juice perhaps through the development of another off-note that obscured the sweet note.

Changes in phenol content on storage

The total phenol content of the juices was not greatly changed by long term storage. There was a notable drop between 1 and 2 weeks of storage at either temperature (Fig. 6) but then there was only a slow decline with time.

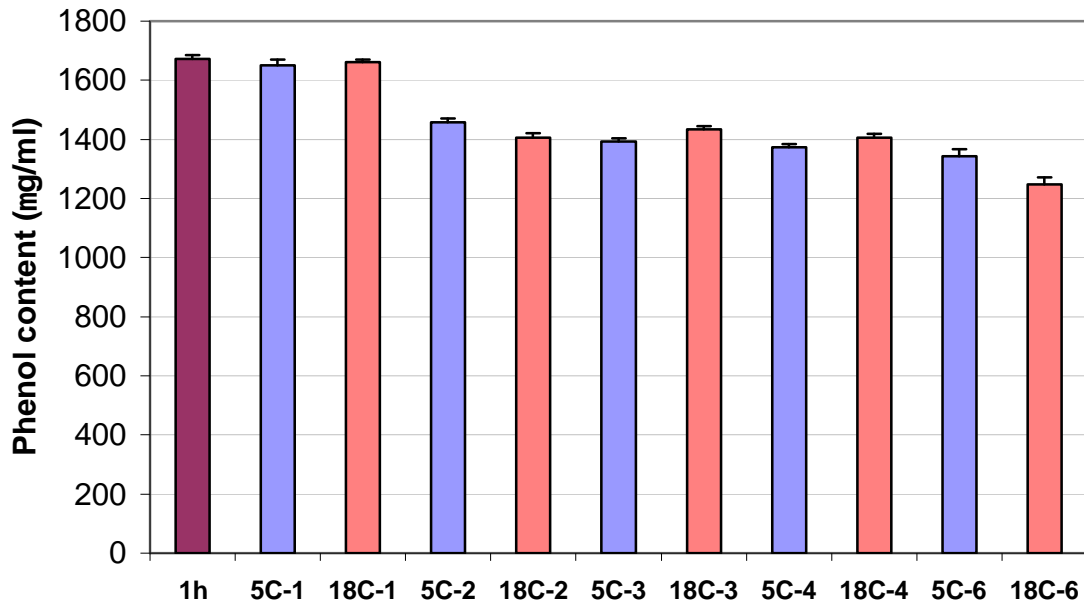


Fig. 6 Effect of storage on total phenol content

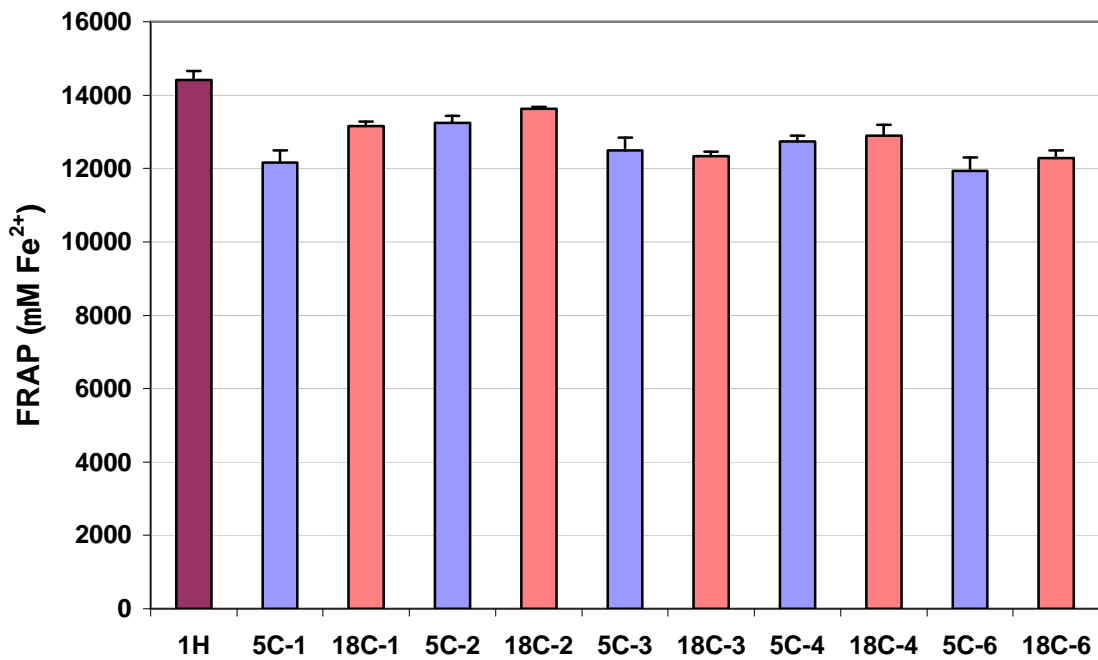


Fig. 7 Effect of storage on total AOC (FRAP)

The AOC does not strictly follow the total phenol content but gives the same general pattern – reduction between 0 and 1 week then little significant decline.

On the other hand, there was a pronounced effect of storage temperature on anthocyanin content (Fig. 8). It declined slowly with storage at 5°C but storage at 18 °C accelerated this decline. Therefore, after six weeks storage at 5°C, ~80 % of original anthocyanin content remained whereas storage at 18 °C retained < 40 %. The LC-MS results also reveal this degradation of anthocyanins at 18°C and suggest differential stability of different individual anthocyanins.

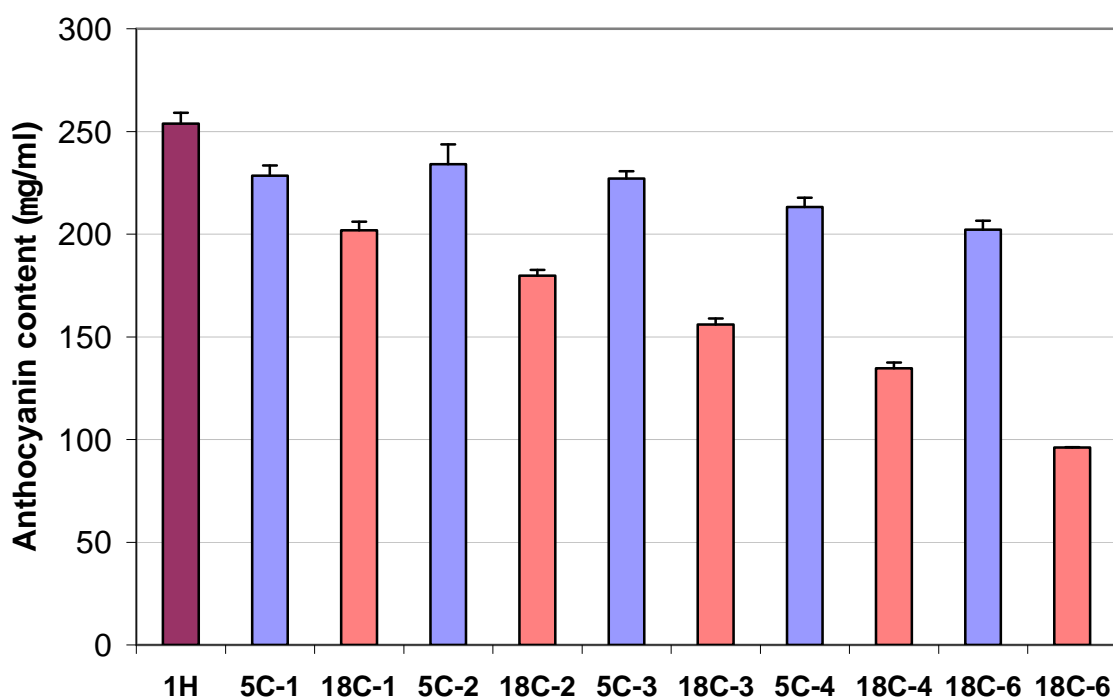


Fig. 8 Effect of storage on total anthocyanin content

It should be noted that the likely products of anthocyanin degradation are phenolic compounds and these would react with the Folin reagent. This would explain how anthocyanin content can fall so much without a corresponding decrease in phenol content. The degradation products were not obvious in the LC-MS analysis (see Annex).

There was a notable decline in vitamin C content within the first week of storage at 18 °C (Fig. 9). The vitamin C levels showed more variation than other parameters but it was clear that vitamin C content seemed relatively well preserved. For example, the rate of decline at 5°C was less pronounced and at six weeks, both juices retained a large portion of their original vitamin C content. *Obviously, any claim on the vitamin C content of the final juice product would depend on the formulation and would have to be based on the selected shelf life of the final product.*

The protection of vitamin C content in juices with high phenolic content is well known and there have been noted interactions between anthocyanin degradation and retention of vitamin C content, presumably by sacrificial protection.

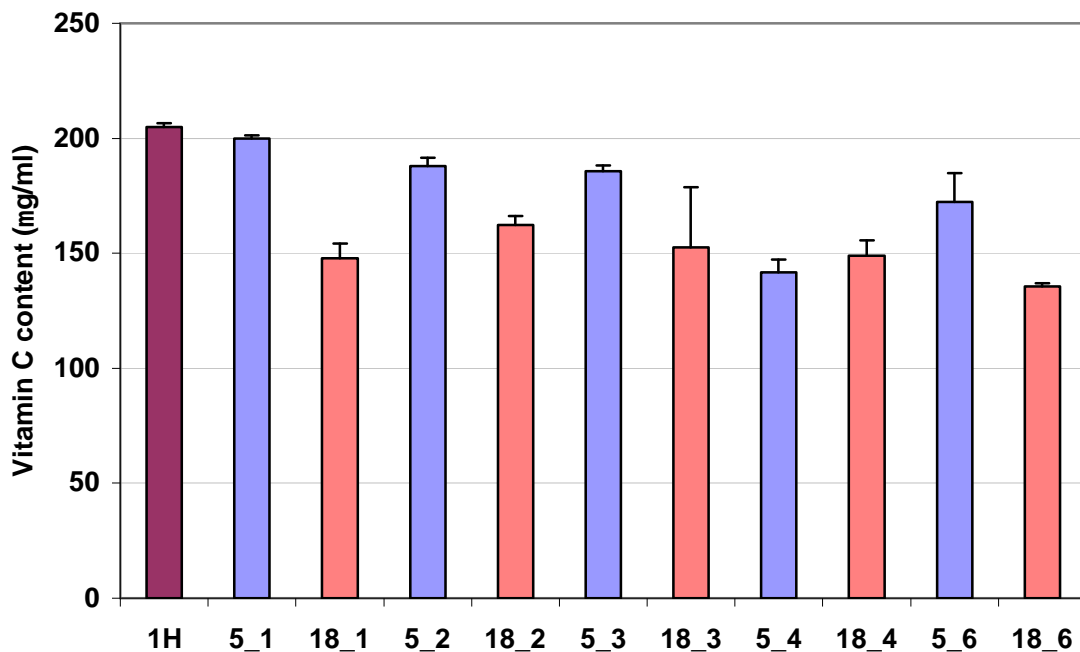


Fig. 9 Effect of storage temperature on vitamin C content

Source juices

The total phenol, anthocyanin and FRAP values were assessed for a range of source juices. These were AP = Aronia pasteurised, AS = Aronia steamed, BC = blackcurrant, RB = raspberry, RC = red currant, SB = strawberry and PGJ = purple grape juice.

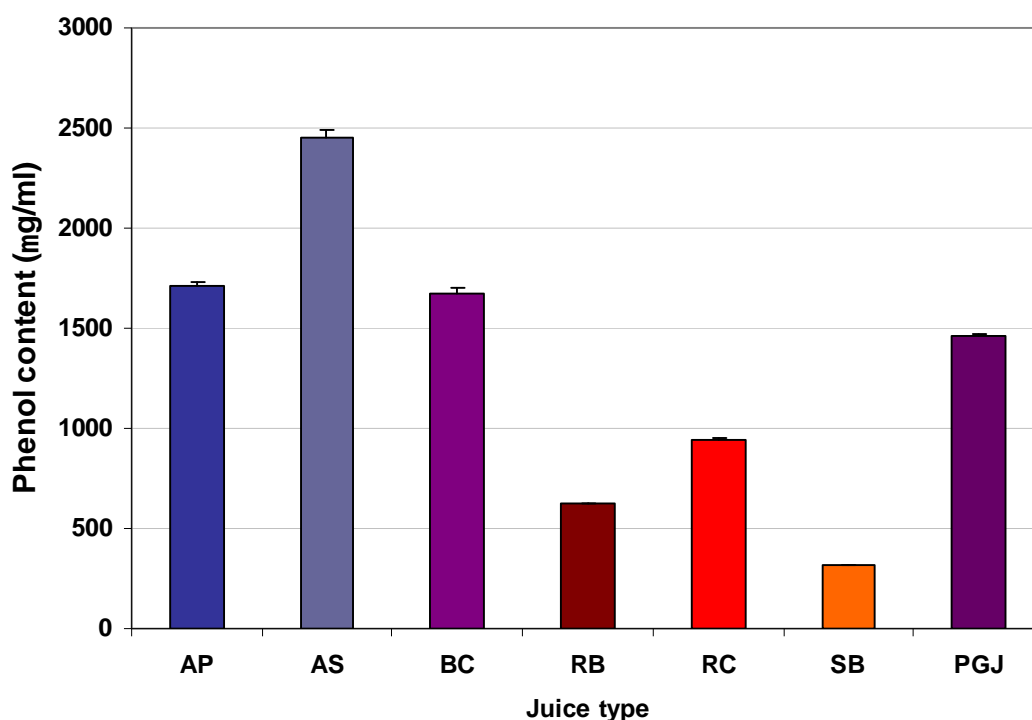


Fig. 10 Total phenol content of juices

The juices showed a wide range of total phenol contents and these were largely reflected in their antioxidant capacity values (FRAP).

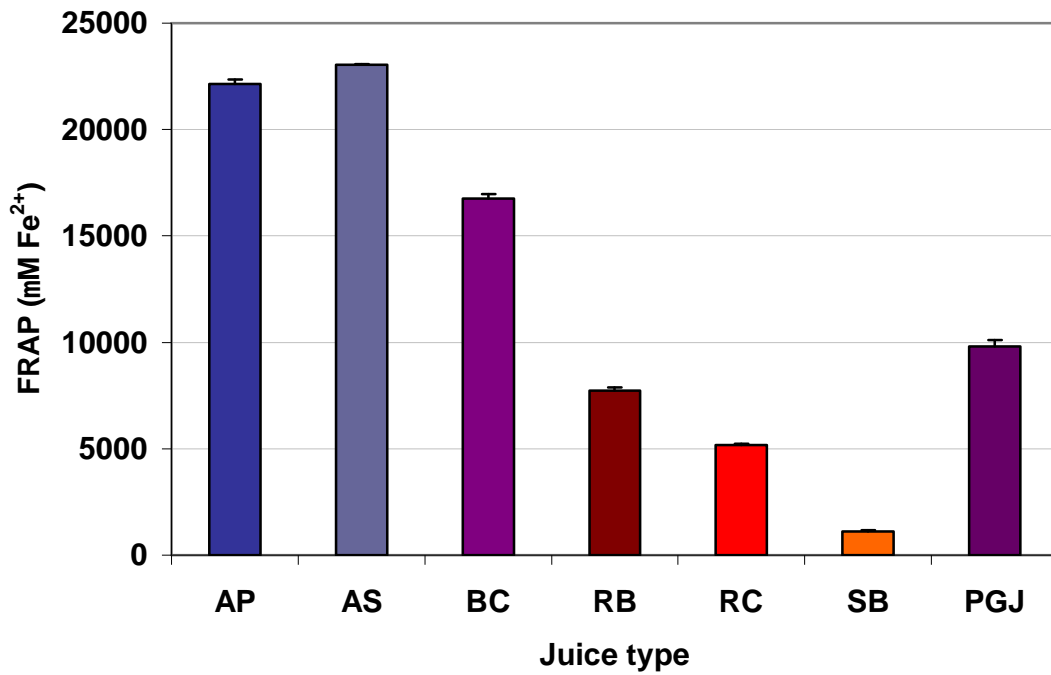
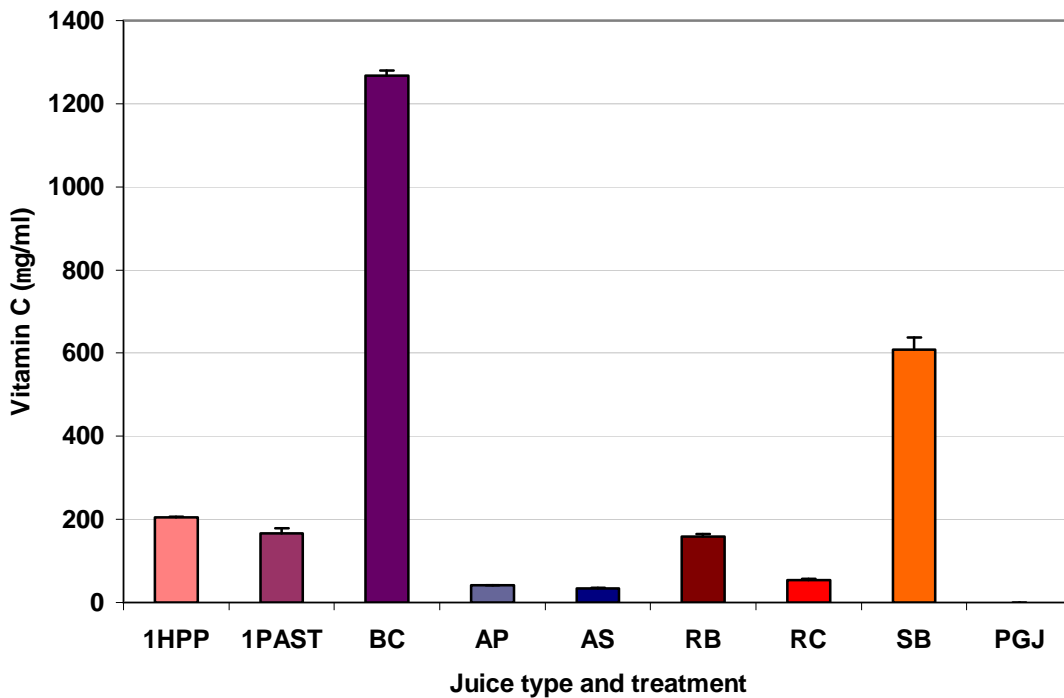


Fig.11 Antioxidant capacity of different juices (FRAP)



The vitamin C contents of the original juices show the source of vitamin C in the juice mixture. Black currant and strawberry had the highest contents.

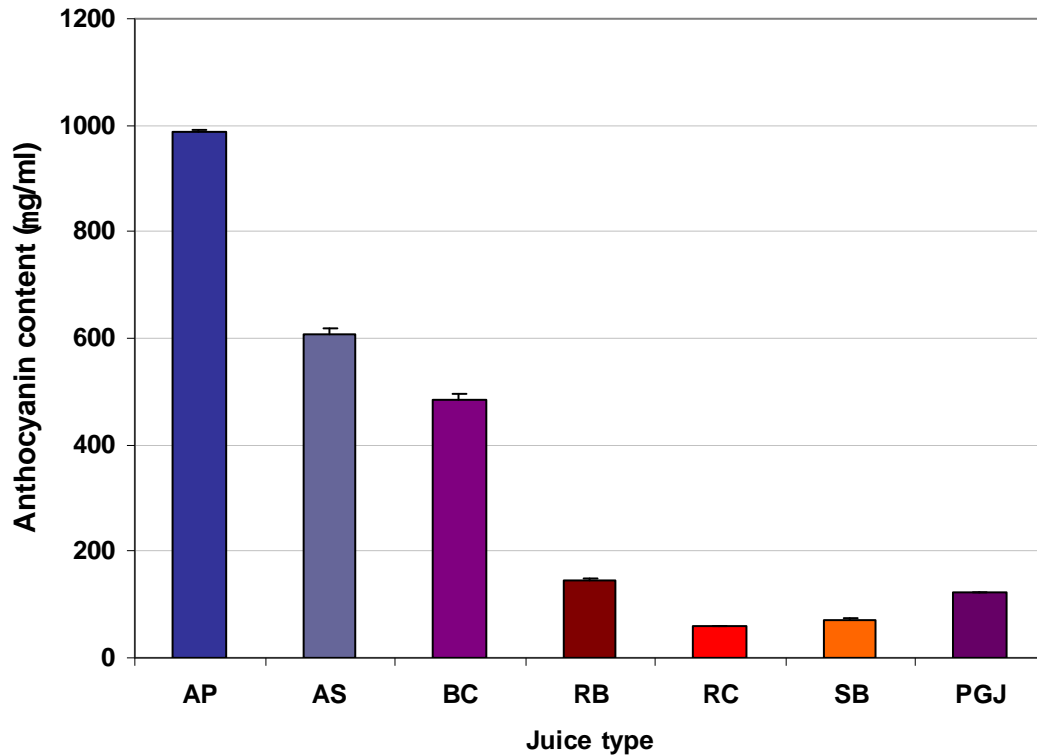


Fig. 13 Total anthocyanin content of juices

The juices were widely different in their total anthocyanin content with Aronia juice pasteurized higher than the others. AP was considerably higher than the AS which suggests some anthocyanin degradation during steaming compared to HPP treatment. This is of interest and we would be willing to follow this up for a potential research paper.

Compositional analysis by HPLC-MS largely backed up these findings (see Annex data).

METHODS

Juice mixtures, source juices and purees were received in a frozen state from the supplier and stored frozen until the storage experiments could be started. Individual samples for each storage temperature and timepoint were used. The commercial comparator juice was also frozen to maintain equivalence with the other juices. Samples were either stored in the dark at room temperature (average day temperature 18°C; high maximum 22°C, low minimum 14°C) or in the cold store (5°C ± 1°C). Samples were mixed by inversion on each work day.

Juices were clarified by centrifugation (16 000 g, 10 min, 5°C) before analysis. Total dry matter was estimated by freeze-drying the pellets in pre-weighed tubes from 6 replicates and reweighing them.

Antioxidant capacity (by the FRAP method), total phenol content and anthocyanin content were measured as described by Deighton et al. (2000). All values shown are averages of triplicate assays ± standard errors.

Liquid-Chromatography Mass Spectroscopy (LC-MS) analysis was carried out as described by McDougall et al., (2008). Components were identified from literature data and through previous work at SCRI.

Total vitamin C content was measured by the HPLC method of Walker et al (2006).

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