SCREENING OF RASPBERY FRUIT FOR CAROTENOIDS: IMPACT ON FLAVOR AND COLOR

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Introduction:

The red, purple, or black color of raspberries is from water soluble pigments, the anthocyanins. Tiny amounts of the fat soluble pigments, the carotenoids, are present in all raspberry fruit and give the yellow color to yellow raspberries. The carotenoids provide the basis of much of the flavor in raspberries, and the balance of compounds such as ionones and fruity esters give a range of flavors among varieties and environments. Key carotenoids thought to be linked to flavor include beta and alpha carotene.

Approach:

Raspberry varieties tested were Caroline, Autumn Britten, Nantahala, and Nova. Fruit from 2010 and 2011 harvests were freeze dried, powdered, and filtered to remove seeds. About 0.4 gms extracted with 10 mls hexane, four mls taken to dryness under nitrogen gas then redissolved in one ml hexane, filtered, and injected onto a Hitachi HPLC (high performance liquid chromatograph) equipped with photodiode array using a gradient of isopropanol, water, and methanol and a C30 4.6 x 250 mm YMC/Waters column. Wavelengths of 248, 470, 325, 292 were used to detect ultraviolet-absorbing compounds (tochopherols), visible light absorbing (carotenoids) and cis isomers. External standards of beta and alpha carotene, beta cryptoxannin, and tocopherol were used to quantify peaks. Each sample was run in triplicate. To clarify apparent carotenoid esters, saponification (the process of breaking fatty acids from carotenoid structures) was done using a 30 minute contact of dried powder to 10% potassium hydroxide, then washed, dried, and resuspended in hexane.

Summary of Results:

Caroline and Autumn Britten are low flavor fruit when grown in North Carolina, while Nantahala is high flavor and Nova has almost no flavor. Also, Caroline and Autumn Britten develop very dark color (dark red) while Nantahala and Nova are light colored (light red to red).

Carotenoids lutein, zeaxanthin, alpha carotene, beta carotene, and beta cryptoxanthin were identified by spectra, retention time, and quantified using standards (Fig 1, Table 1). Alpha cryptoxanthin, not reported previously in raspberry, also appears to be present but will have to be verified using mass spectrophotmetry as no standard is available. Alpha carotene content was higher than that of beta carotene in all raspberry varieties. In contrast, Apache blackberry had very high levels of beta carotene compared to raspberry.

Both alpha and beta carotene were low in Nova raspberry. Nova had a number of peaks in the non polar area of separation, which, when saponified, were found to be esters of alpha and beta carotene and tentatively, alpha and beta cryptoxanthin (Figs 2,3). This accumulation indicates that Nova can synthesize and hold onto carotenoids, but may accumulate less carotenoids than the other raspberries.

The low levels of alpha and beta carotene in Nantahala, a high flavor raspberry, compared to the carotenes in the low flavor Caroline and Autumn Britten may indicate that Nantahala is converting these carotenes into the ionone flavor compounds at a faster rate than the other varieties.

While alpha and beta carotene are the basis of strong flavor impact compounds of alpha and beta ionones, the other carotenoids (lutein, alpha and beta cryptoxanthin isoprenoids) provide grassy notes. We have not finished looking at raspberries of the same varieties grown under differing environments; we may find that isoprenoids become more concentrated with heat.

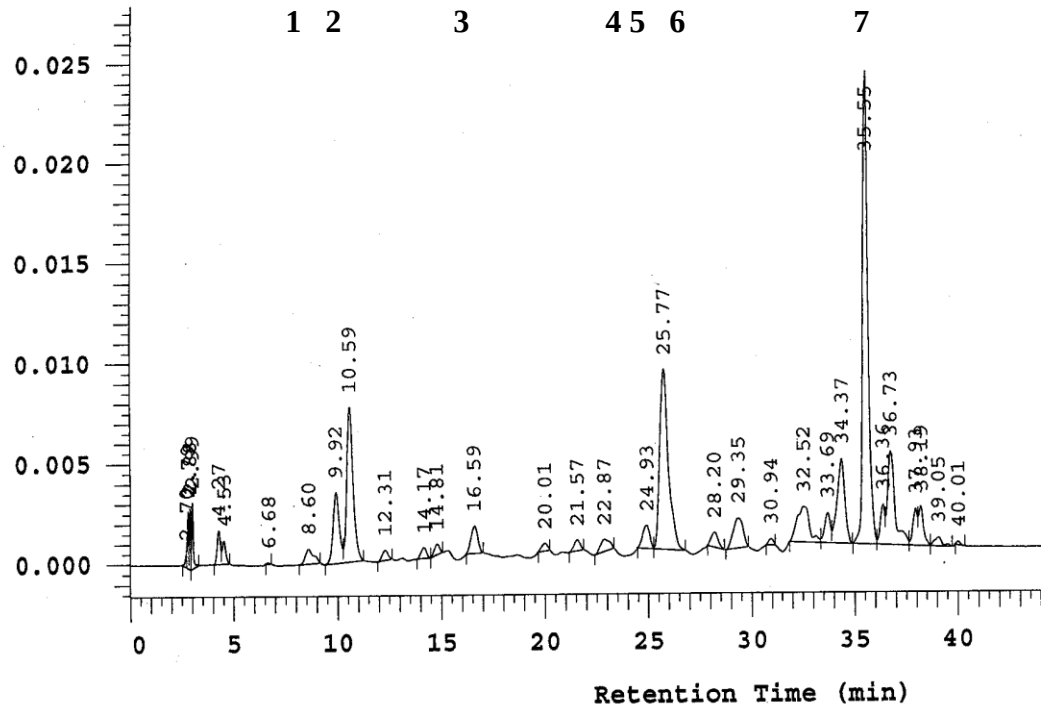
Conclusions:

In this project, we were able to quantify carotenoids in four raspberry varieties. We have developed a protocol that can be used to follow raspberry carotenoids in selections of interest, and believe that the carotenoid profiles in raspberry will lead to a much more complete picture of how genetic and environmental changes affect raspberry flavor.

Table 1. Carotenoids quantified in raspberry and blackberry from freeze dried berries with seeds removed (units as micrograms per 100 gms dry weight).

Raspberry variety	Lutein	a-carotene	b-carotene	total carotenoids
Nova	13.89	3.42	1.42	18.73
A. Britten	64.55	41.09	18.49	124.13
Caroline	91.08	46.99	21.44	159.51
Nanatahala	29.27	21.31	8.68	59.26
Apache blackberry	27.79	19.17	137.17	184.13

Figure 1. ‘Nantahala’ HPLC profile



Peak:

1. **Lutein**
2. **Zeaxanthin**
3. **a-cryptoxanthin**
4. **a-carotene**
5. **a-carotene ester**
6. **b-carotene**
7. **lutein ester**

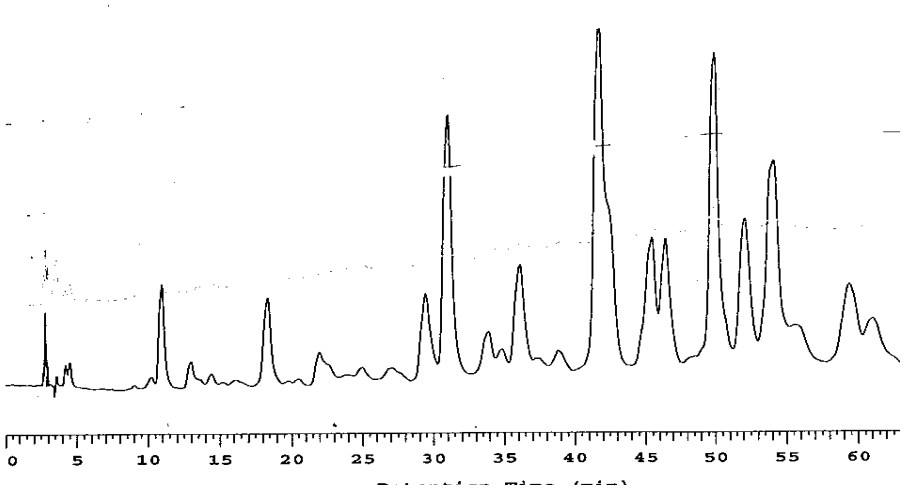


Figure 2. Nova raspberry carotenoid profile before saponification. Peaks to right side are more non-polar (less water soluble).

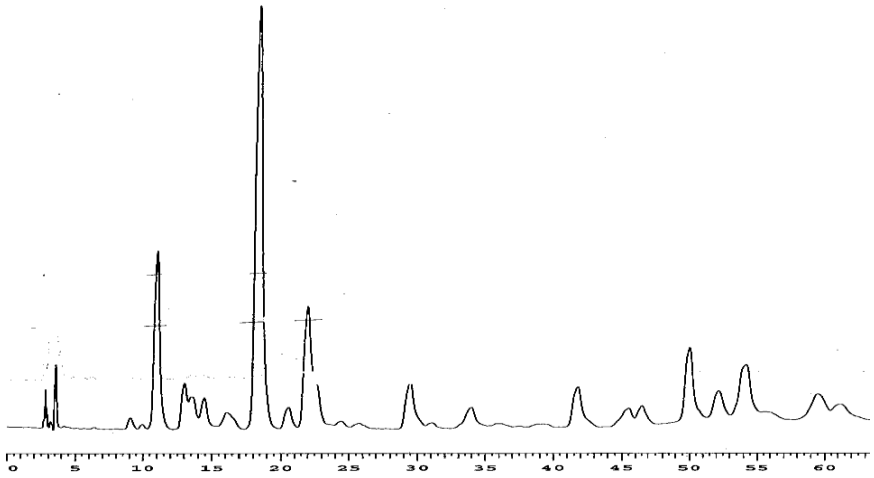


Figure 3. Nova raspberry carotenoid profile after 30 minute saponification. Note loss of peaks on right side and increase of peak height on left side.