

# Biosynthesis of carotenoids during bilberry (*Vaccinium myrtillus* L.) fruit development

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## Background

Bilberry (*Vaccinium myrtillus* L.) fruits are rich with anthocyanin pigments but they also contain other bioactive compounds, such as carotenoids. The biosynthesis of carotenoids in plants is well established and takes place in plastids by enzymes that are nuclear-encoded (Cazzonelli and Pogson 2010, Hannoufa and Hossain 2012). Carotenoid biosynthesis pathway is shown in Fig. 1.

## Materials & Methods

The biosynthesis of carotenoids was studied in different stages during bilberry fruit development and ripening and as response to light treatments. Eight key carotenoid biosynthetic genes (*PSY*, *PDS*, *ZDS*, *CRTISO*, *LCYE*, *LCYB*, *BCH*, *CYP450-BCH*) as well as a carotenoid cleavage dioxygenase class 1 (*CCD1*) were isolated from bilberry and their expression was analyzed with qRT-PCR. Composition of carotenoids was analyzed from the same samples with HPLC-MS.

## Results & Discussion

The most abundant carotenoids in bilberry fruit were lutein and  $\beta$ -carotene accompanied by minor amounts of xanthophylls such as neoxanthin, violaxanthin and zeaxanthin (Fig. 2). The expression of the carotenoid biosynthesis genes showed increase in transcript levels of phytoene synthase (*VmPSY*), phytoene desaturase (*VmPDS*), carotenoid isomerase (*VmCRTISO*) and lycopene  $\beta$ -cyclase (*VmLCYB*) at the onset of fruit ripening (Fig. 3). However, the increase in the expression did not lead to the accumulation of carotenoids during ripening in ripe berries and the carotenoid levels decreased during the fruit development. Increased expression of the *VmCCD1* during the ripening stages indicates cleavage of carotenoids to apocarotenoids such as abscisic acid (ABA) (Fig. 3). Our earlier study on ABA biosynthesis in bilberry showed similarly increased expression of *VmNCED1* during the ripening stage (Karppinen et al. 2013). Light conditions during fruit development were found to affect to the expression of *VmPSY*, *VmPDS*, *VmCRTISO*, *VmLCYB* and *VmLCYE*, especially ripe fruits (Fig. 4).

## Conclusions

The results suggest that the carotenoid levels in bilberry fruit are determined by their biosynthesis and degradation of carotenoids to apocarotenoids such as abscisic acid. Red light increases the expression of the carotenoid pathway genes in ripe bilberry fruits.

## References

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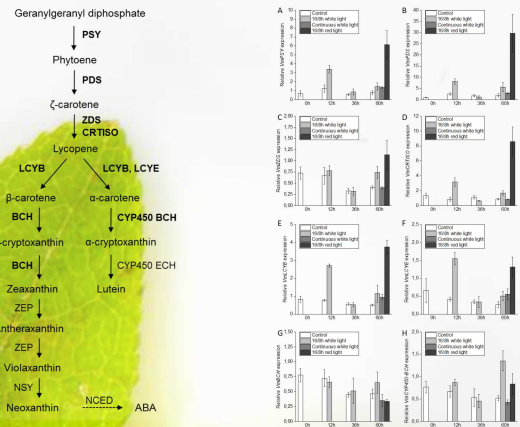


Fig. 1. The carotenoid biosynthetic pathway in higher plants (Stigliani et al. 2012). PSY, phytoene synthase; PDS, phytoene desaturase; ZDS,  $\zeta$ -carotene desaturase; CRTISO, carotenoid isomerase; LCYB, lycopene  $\beta$ -cyclase; LCYE, lycopene  $\epsilon$ -cyclase; BCH,  $\beta$ -carotene hydroxylase; CYP450-BCH, carotenoid  $\beta$ -ring hydroxylase of cytochrome P450 family; ZEP, zeaxanthin epoxidase; NSY, neoxanthin synthase

Fig. 4. Effect of light on carotenoid biosynthesis in ripe berries. The relative expression of the genes was quantified by qRT-PCR and normalized to *VmGAPDH*. Values represent means  $\pm$  SE of three replicates.

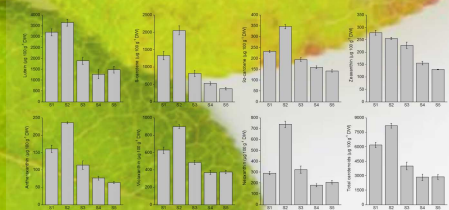


Fig. 2. Content of carotenoids ( $\mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ ) during bilberry fruit development. Values represent means  $\pm$  SE of four replicates. S1 = flower, S2 = small unripe green berry, S3 = unripe green berry, S4 = red ripening berry, S5 = ripe berry.

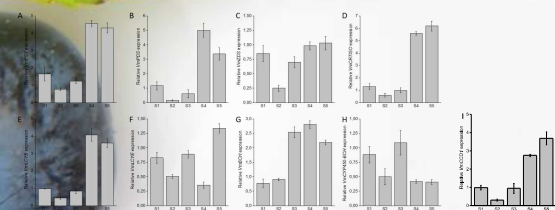


Fig. 3. Expression of carotenoid biosynthetic genes (A) *VmPSY*, (B) *VmPDS*, (C) *VmZDS*, (D) *VmCRTISO*, (E) *VmLCYB*, (F) *VmLCYE*, (G) *VmBCH*, (H) *VmCYP450-BCH* and (I) *VmCCD1* during bilberry fruit development. S1–S5 indicate the fruit developmental stages from flower to fully ripe berry. The relative expression of the genes was quantified by qRT-PCR and normalized to *VmGAPDH*. Values represent means  $\pm$  SE of at least three replicates.

