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Pigmentation "clue" in Yellow Raspberries - Carotenoids and masking effect of anthocyanins

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Fruit pigmentation in raspberries (*Rubus idaeus* L.) is a complex phenomenon and one of the most important traits where a range of colour patterns from deep purple to yellow exists. Anthocyanins and carotenoids are known to be the main pigments, while colour of fruits is mainly considered to be due to varying anthocyanin contents. However, carotenoids seem to be responsible for the yellow colour and they are just masked by anthocyanins in the case of red varieties. Even though apocarotenoids, carotenoid breakdown products, are responsible for the characteristic raspberry flavour, and although these compounds seem to have very beneficial effects in health, raspberries carotenoid composition and carotenoid biosynthesis in raspberry has been very little studied.

To better understand the origin of yellow colouration observed in raspberry varieties, targeted metabolomics approaches focusing on carotenoid and (poly)phenolic pattern were performed (1,2). The composition of carotenoids, chlorophyll derivatives and tocopherols in raspberries of different varieties, including yellow and red varieties, was studied during a STSM (EC at RHUL) over different ripening stages. The profile of pigments in ripening raspberries changes drastically, with a dramatic decrease of β-carotene and chlorophyll derivatives, the xanthophyll lutein has also decreased but not to the same extent. In contrast lutein esters increased and are present in ripe raspberries esterified with saturated fatty acids with C8 to C16 chains. Ripe raspberries contain considerable amounts of lutein, lutein esters, and tocopherols (up to 20, 49 and 366 mg/kg dry weight, respectively). As a result, the different samples analysed show different contents of carotenoids and tocopherols. Whether the differences arise from the variety or other factors such as the environmental conditions needs to

be ascertained but isoprenoids should not be neglected when considering raspberry antioxidant and nutraceutical composition.

Fruit ripening in yellow raspberries is associated with biosynthesis of different amounts of carotenoids in the biosynthetic pathway. Genetic and functional analysis of the pathway genes will further elucidate the important role of carotenoid pigments in raspberry. There is also possibility of mutations in the pathway that may indicate reason for different amounts of carotenoids and various fruit color shades in yellow raspberries. For functional characterization of putative carotenoid pathway genes from *Rubus* in microbial host (STSM of MZR at RHUL) we took advantage of an in house preliminary draft of the raspberry genome to identify homologous carotenoid pathway genes and to clone candidate genes into standard expression cassettes. These plasmids were used to complement with plasmids capable of generating different carotenoid precursors. The co-transformation results in a visible colour change in the *E. coli* host, indicating thefunctional assignment. Upon the detection of visual colour, analysis of carotenoids was carried out to ascertain the carotenoids present. In the case of carotenoid cleavage enzymes the visible screen is the reduction in colour compared to the precursor line.
Based on these results a pathway map for pigmentation in raspberry will be predicted and utilized for biotechnological production of specific carotenoids and aroma compounds.