Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.11

PURIFICATION AND PROPERTIES OF *MALUS DOMESTICA* CAROTENOID CLEAVAGE DIOXYGENASE 4

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Carotenoid bleaching, functional food, Malus domestica, Prunus persica, substrate range

During recent years increasing evidence supporting the ability of the so-called functional foods to promote human well-being and reduce the risk of certain major diseases prompted a strong interest for the development of strategies to increase the levels of health-promoting bioactive compounds in fruits and vegetables. To ensure protective effects, relatively high levels of phytonutrients would be in fact required, well over those commonly taken with the dietary consumption of plant-derived foods. Among these beneficial substances are the carotenoids, powerful antioxidant tetraterpenoids able to protect against oxidative stress by quenching singlet oxygen and scavenging free radicals, therefore inhibiting lipid peroxidation. Besides the health-promoting effects, the presence of these compounds in fruits, vegetables, staple and processed foods is also attractive for the consumer. The yellow-to-orange colour rendered by high carotenoid content often increases buyer confidence and, as a consequence, product value. Typical examples are the pasta products, and the yellow-fleshed peach fruits.

Emerging data suggest a pivotal role of carotenoid-bleaching enzymes in determining the final level of these phytonutrients in foodstuffs. During pasta processing, a loss of colour as a consequence of pigment oxidation usually occurs that is mainly due to the lipoxygenase-linoleate system (Verlotta *et al*., BMC Plant Biology 2010, 10:263). In peach, carotenoid accumulation in the mesocarp causes the difference between yellow and white genotypes. The latter are generally characterized by a peculiar and more intense aroma, because of the formation of volatiles deriving from the breakdown of the carotenoid skeleton. Dioxygenases appear to be key factors causing volatile release in fruits, and a differential expression of *carotenoid cleavage dioxygenase* (*CCD*) *4* gene was in fact found in yellow *vs.* white-fleshed isogenic peach genotypes (Brandi *et al*., BMC Plant Biology 2011, 11:24).

Within the frame of a research project aimed at improving our knowledge on the biochemical bases of carotenoid content in peach fruits, we expressed the *CCD4* gene from *Malus domestica* in a heterologous system. The protein was purified to near electrophoretic homogeneity by sequential affinity chromatography, anion-exchange and gel-filtration FPLC. Because carotenoid breakdown by CCDs yields a coloured product, an HPLC-based assay method was set up to measure *in vitro* carotenoid cleavage reactions. However, the isolated enzyme showed an almost negligible activity toward either β-carotene or trans-β-apo-8'-carotenal, suggesting a possible cofactor requirement. Activity assay performed with extract from induced *vs.* not-induced *E. coli* transformed strains provided evidence of cleavage activity. However, contrary to previous data (Huang *et al*., J. Exp. Bot. 2009, 20:3011), a higher affinity was found toward trans-β-apo-8'-carotenal than β-carotene.

Experiments are currently in progress in order to raise polyclonal antibodies in mice against the purified apple CCD4. In the next future these will be used as a tool in characterizing protein

levels in tissues at different stages during fruit development in contrasting genotypes of peach showing various levels of carotenoids in fruit flesh.

This work was supported by a grant from the Italian MiPAAF in the frame of the ALISAL project.