

## LIPOXYGENASE ACTIVITY IN *TRITICUM DURUM*: DIFFERENTIAL PROPERTIES OF ENZYME FORMS

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Durum wheat (*Triticum turgidum* L. ssp. durum) is the main cereal crop in the Mediterranean area. It is used to produce pasta, for which a proper yellow colour is an important quality parameter that increases buyer confidence and product value. The colour is given by the presence of carotenoid pigments in semolina, mainly lutein. However, a significant loss of these pigments may occur during pasta processing as a consequence of the activity of lipoxygenases (LOX, linoleate:oxygen oxido-reductase, EC 1.13.11.12). The hydroperoxides produced generate in turn volatile compounds, which give undesirable flavour to foodstuff. The presence of lutein is not only an aesthetic parameter of pasta products, but has also a nutritional value. Carotenoids in the diet reduce the risk of ocular diseases, including the age-related macular degeneration, and have been recently related also to an increased protection from developing cardiovascular diseases and several types of cancer. Therefore the enhancement of lutein content in durum wheat may produce beneficial effects on human health, particularly for those populations for which the daily intake of carotenoids is well below the recommended dose.

Lipoxygenases are non-heme iron-containing dioxygenases present also in microorganisms and animals. Their substrate in plants is represented mainly by linoleic, linolenic and arachidonic acids. Several enzyme forms showing distinct biochemical properties (pH optimum, substrate range and affinity, product stereospecificity) have been reported in several species. Two isoenzymes have been purified and characterized in barley (Holtman *et al.*, Plant Physiol. 111, 569-576, 1996). LOX1 produces mainly 9-hydroperoxide-octadecadienoic acid, whereas LOX2 converts linoleic acid into 13-hydroperoxide-octadecadienoic acid. LOX-1 is present in both quiescent and germinating seeds and is responsible for most activity in mature grains, whereas LOX-2 is found only at early stages of grain development and after germination. Specific features suggest specialized functions, yet the role of each isoform has not been fully elucidated.

Within the frame of a research project aimed at improving our knowledge on the biochemical bases of carotenoid content in durum wheat and pasta products, LOX activity was characterized in *T. turgidum* ssp. durum cv Ofanto. Recent data at the molecular level showed the presence in this species of no less than five genes with different allelic variants, some of which were associated with a strong reduction in LOX activity in semolina (Verlotta *et al.*, BMC Plant Biology 2010, 10:263). However, no direct evidence of a differential catalytic rate has been obtained to date. Two isoforms were resolved in extracts from suspension cultured cells by ion-exchange liquid chromatography. Biochemical characterization carried out with three different assay methods showed diverse functional properties, coupled with a distinct pattern of expression during the cell culture growth

cycle. Experiments are in progress in order to purify isoforms to electrophoretic homogeneity, and to investigate their carotenoid bleaching activity.

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