

Molecular Characterization of Aroma Biogenesis in Olive Fruit: cDNA Cloning and Expression Studies of a Hydroperoxide Lyase Gene

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Virgin olive oil aroma is the result of a complex mixture of volatile compounds synthesized during the crushing and malaxation steps within the olive oil production, and mainly characterized by green odor notes due to aldehydes and alcohols of six straight-chain carbons (C6) and to the corresponding esters. These compounds are synthesized from polyunsaturated fatty acids containing a (Z,Z)-1,4-pentadiene structure such as linoleic and linolenic acids by the action of the lipoxygenase (LOX) pathway. In a first step, LOX activity produces the 13-hydroperoxide derivatives from polyunsaturated fatty acids, that are subsequently cleaved by hydroperoxide lyase (HPL) to C6 aldehydes, which can undergo reduction by alcohol dehydrogenases (ADH) to form C6 alcohols. Finally, alcohol acyltransferase (AAT) activity transforms C6 alcohols into the corresponding esters.

Continuing with the molecular characterization of the enzymes involved in the LOX pathway in olive fruit, we have isolated a full-length cDNA clone encoding a hydroperoxide lyase (*OepHPL*) from an olive fruit (cv. Picual) cDNA library, using a PCR approach. The deduced amino acid sequence contained an N-terminal plastidial transit peptide. Alignment of the sequence revealed a high degree of identity to known plant HPL sequences and the phylogenetic analysis showed that is grouped with HPL enzymes that exhibit 13-HPL activity. The *OepHPL* gene has been also functionally expressed in bacteria, and the recombinant enzyme purified and biochemically characterized. Furthermore, expression analysis was carried out using distinct olive fruit tissues and at different developmental stages. The molecular and biochemical properties of this HPL gene/enzyme are consistent with an important role in the aroma biogenesis in the olive oil production.