

# Biochemical and structural characterization of a newly identified lipase in hazelnut (*Corylus avellana*)

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Rancidity is common in oilseeds. Although the molecular basis of this process have not been clarified in full, lipases play essential role in the release of free fatty acids (FFA) that reportedly turn rancid much faster than triacylglycerols (TAG)[1]. From the industrial standpoint, the identification and characterization of lipases as potential “predictive” markers of raw material shelf-life seems relevant to making food processing and storage more sustainable and efficient[2] as well as for climate change effect on seed development. A new protein purification protocol, combined with lipolytic enzymatic assay, was set up to isolate lipase(s) from hazelnut, and led to the identification of a monomeric 9 kDa protein active towards both synthetic and natural substrates, with optimal activity at 37°C and pH 8.0. Surprisingly, this novel enzyme shares little if any sequence or structure homology with canonical lipases. Structural characterization through circular dichroism reveals a predominantly  $\alpha$ -helical structure. It should be noted that 40% of the signature spectroscopic features of the protein’s secondary structure were still present after a temperature-ramp treatment from 20 to 90 degrees. The unusual stability of the secondary structure of the enzyme may stem from the intrinsic rigidity conferred by the fact that it contains 8 cysteine residues paired to form 4 disulfide bridges (thermal stability is drastically reduced in the presence of reductants), similarly to what observed for other plant proteins involved in lipid transfer and metabolism in plants.

[1] Rosso MC et al. (2018) Analytical and Bioanalytical Chemistry 410,15: 3491-3506.

[2] Li, Bo et al. (2016) PloS One 11,12 e0167330.