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## OPTIMISATION OF GLYCOSIDASES FOR RELEASE OF AROMAS FROM STRAWBERRY GLYCOSIDES

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In fruits a portion of potentially aromatic molecules are bound to sugars, so that they are not volatile and do not contribute to aroma. Glycosidases are a promising way to release this aromatic potential in fruits, especially in strawberries, and thus offer a solution to limit addition of (synthetic) aromas in fruit products. However, these enzymes are usually sensitive to the concentration of sugars and to low pH. Fruits are therefore not the most adapted media for these enzymes. An optimization of glycosidases by enzyme engineering was therefore carried out with two main aims. The first is to increase their activity in the fruit conditions and the second is to limit side activities that may affect color and texture of fruit products. Indeed, anthocyanins in strawberries are glycosylated and the aglycones lose color rapidly, and the commercial enzyme used for glycosidic activity has also a pectinolytic activity which destroys the texture of fruit purees. The Shuffling® method was selected to decrease the sensitivity to sugars and to increase activity. After screening two new enzymes are kept, one (F1) with a low sensitivity to sugars and another (F2) with a higher specific activity. F1 lost only 27% of its activity in a sugar solution (18% of fructose, 16% of glucose and 11% of sucrose) instead of 80% for the parental enzymes, and F2's specific activity was increased by 50% compared to parental enzymes. Texture and color were not affected by the new enzymes. Different concentrations were tested for an incubation time of 90 minutes and for a temperature of 40°C (optimal temperature of engineered enzyme) to identify minimal required enzyme concentrations. Enzyme denaturation by pasteurization was also studied to ensure that the new enzymes will belong to processing aids.