Chalcone Synthase: Method Optimization of Extraction and Activity Assay in Strawberry Fruit

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ABSTRACT

Method development and optimization play a central role in analytical chemistry and more specifically in food biochemistry. When it comes to research, it is common that analytical methods need to be modified to specific experimental biological tissues. While there are several published works on the activity of the enzyme chalcone synthase (CHS) in plant materials, such as sweet basil using ultra-high-performance liquid chromatography, there is a lack of information regarding extraction and activity of CHS in strawberries. Therefore, the main objective of this work was to optimize existing published methods for extraction and activity of CHS in strawberries, using spectrophotometric analysis. It was done through a literature search, a method dissection was performed, followed by theoretical optimization of the protocol, and finally a experimental optimization.

INTRODUCTION

Chalcone synthase is a ubiquitous enzyme in higher plants. It catalyzes the sequential decarboxylative addition of three acetate units from malonyl-CoA to a *p*-coumaroyl-CoA starter molecule deriving from phenylalanine through the phenylpropanoid pathway¹, which is a rich source of metabolites in plants². While there are several published works on the activity of the enzyme chalcone synthase (CHS) in plant materials, such as sweet basil using ultra- high-performance liquid chromatography, there is a lack of information regarding extraction and activity of CHS in strawberries. From the literature search we based our method optimization on the work published by Ghasemzadeh et al. (2016) on sweet basil using spectrophotometry and ionic exchange resin rather than HPLC. This protocol was analyzed and adjusted for strawberry fruit.

Fig 1: Structure, CoA binding, and overall reaction of chalcone synthase (CHS). Each monomer are colored in gold and blue, the bottom panel shows the overall reaction catalyzed by CHS with the malonyl derived portions of chalcone shown in red (Austin et al., 2003)

MATERIAL AND METHODS

Strawberry fruit will be homogenized and 0.4 g of fruit puree will be mixed with a solution of 40 mg of 2-mercaptoethanol dissolved in 500 mL of 0.1 M borate buffer at 4 °C.

Borate Buffer will be prepared as follows:

- 3.1 g of boric acid plus 300- 400 mL of D.I. water
- 1 mol of NaOH to adjust the pH to around 8

0.1 g of Dowex I × 4 resin will be added to the solution, for ion exchange and lower the reaction time

To the supernatant 100 mL of 10 mM of potassium cyanide and 500 mL of 1 M of Tris-HCl buffer will be added.

10 mg of chalcone will be added to 10 uL ethylene glycol monomethyl ether, mixed with enzyme extract, and left at for 1 min at 30 °C.

The absorbance will be measured at 370 nm.

The final protocol will be tested, with some possible further modification, such as adjustment of sample size, the volume or the concentration of each solution and temperature.

Fig 2: Phenylpropanoid metabolic pathway. Enzyme activity and interaction present in the pathways (Zabala et al., 2006)

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