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Research Paper

The use of tomato aminoaldehyde dehydrogenase 1 for the detection of aldehydes in fruit distillates

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Plant NAD$^+$-dependent aminoaldehyde dehydrogenases (AMADHs, EC 1.2.1.19) belong to the family 10 of aldehyde dehydrogenases. They participate in the metabolism of polyamines or osmoprotectants. The enzymes are characterized by their broad substrate specificity covering ω-aminoaldehydes, aliphatic and aromatic aldehydes as well as nitrogen-containing heterocyclic aldehydes. The isoenzyme 1 from tomato (*Solanum lycopersicum*; SlAMADH1) oxidizes aliphatic aldehydes very efficiently and converts also furfural, its derivatives or benzaldehyde, which are present at low concentrations in alcoholic distillates such as fruit brandy. In this work, SlAMADH1 was examined as a bioanalytical tool for their detection. These aldehydes arise from fermentation processes or thermal degradation of sugars and their presence is related to health complications after consumption including nausea, emesis, sweating, decrease in blood pressure, hangover headache, among others. Sixteen samples of slivovitz (plum brandy) from local producers in Moravia, Czech Republic, were analyzed for their aldehyde content using a spectrophotometric activity assay with SlAMADH1. In all cases, there were oxidative responses observed when monitoring NADH production in the enzymatic reaction. Aldehydes in the distillate samples were also subjected to a standard determination using reversed-phase HPLC with spectrophotometric and tandem mass spectrometric detection after a derivatization with 2,4-dinitrophenylhydrazine. Results obtained by both methods were found to correlate well for a majority of the analyzed samples. The possible applicability of SlAMADH1 for the evaluation of aldehyde content in food and beverages has now been demonstrated.

Introduction

Slivovitz (plum brandy) is an alcoholic beverage produced by distillation of fermented plums (*Prunus domestica*) with a typical alcohol content of at least 45% (v/v). This kind of spirits is very popular in Moravia, the eastern region of the Czech Republic. Slivovitz is also produced and very popular in other Central and Eastern European countries such as Bosnia, Bulgaria, Croatia, Hungary, Poland, Romania, Serbia, Slovakia and Slovenia. Similar plum brandies are marketed under different names in France, Germany, Switzerland, USA and Canada [1].

Alcoholic beverages produced by distillation contain various aldehydes at low concentrations. They arise from fermentation processes or a thermal degradation of sugars. The highest concentration is typically found for acetaldehyde (C_2AL), which often exceeds 1.0 mmol l^{-1} [2]. Also other aliphatic saturated aldehydes and furfural (FurAL), 5-methylfurfural (Met-FurAL), 5-(hydroxymethyl)furfural (OH-Met-FurAL), benzaldehyde (BzAL) or acrolein (AcrAL) are commonly present [3,4]. The presence of aldehydes in

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alcoholic beverages is related to health complications after consumption including nausea, emesis, sweating, decrease in blood pressure, hangover headache, etc. [3,5]. The Decree of the Ministry of Agriculture of the Czech Republic no. 141/1997 permits a maximal concentration of FurAL in plum brandy of 50 mg l^{-1} (=0.52 mmol l^{-1}). C_2AL and FurAL can be analyzed as volatiles by gas chromatography [6]. There are also colorimetric and fluorometric methods available for FurAL [7,8].

High performance liquid chromatography with a sample derivatization by 2,4-dinitrophenylhydrazine (DNPH) performed under acidic conditions is used for the detection of aldehydes and ketones in many types of food as well as in medical or environmental samples [3,9,10]. The derivatization of aldehydes and ketones by DNPH was published almost a century ago [11] and already at that time used for their identification [12]. Protocols use hydrochloric, sulphuric or perchloric acid for the acidification of the reaction mixture. Water and methanol or acetonitrile are typical mobile phases [13,14]. After HPLC separation, 2,4-dinitrophenylhydrazones (DNPHOs) of aldehydes can be detected by UV–vis spectrophotometry at wavelengths between 350 and 390 nm [3,10,15] or by mass spectrometry (MS) in the negative mode. Tandem mass spectrometry (MS/MS) allows identification of carbonyl compounds and their isomers by fragmentation of $[M-H]^-$ ions [13,14]. For example, there is an abundant fragment ion with *m/z* 163 registered for DNPHOs of aldehydes, while the fragmentation of the derivatized ketones leads to a fragment ion with *m/z* 179, which contrary to the measurements with aldehydes has a higher intensity than the ion with *m/z* 163. Saturated aldehydes and ketones produce a fragment ion $[M-H-30]^-$, which is not detected in the case of aromatic aldehydes or aldehydes with a double bond in the α-position. Finally, the fragmentation of linear saturated aldehydes leads to a fragment ion with *m/z* 191, which is not well developed for aldehydes with a branched chain [13].

Plant aminoaldehyde dehydrogenases (AMADH, EC 1.2.1.19) catalyze the oxidation of aminoaldehydes to amino acids using NAD^+ as an electron acceptor. They belong to the aldehyde dehydrogenase (ALDH) superfamily as members of the family ALDH10 [16,17]. Plant AMADHs participate in polyamine degradation or production of compatible osmoprotectants [17–19]. Their substrate specificity is very broad. In addition to natural substrates, for example 3-aminopropanal (APAL), there have been many synthetic substrates described: nitrogenous heterocyclic aldehydes (including pyridine, purine and pyrimidine derivatives), bromobenzaldehydes and *N*-acylated-ω-aminoaldehydes [20–23].

The isoenzyme 1 from tomato (*Solanum lycopersicum*), SlAMADH1, has the broadest substrate specificity among all plant AMADHs studied in our laboratory [20,23]. For that reason, we decided to test the possibility of using it as a bioanalytical tool for the detection of aldehydes in fruit distillates. HPLC with a DNPH derivatization of aldehydes was chosen as a standard procedure to validate results from enzyme kinetics experiments. The availability of a fast and simple enzymatic assay of aldehydes applicable without expensive instrumentation would be beneficial for quality control analysis. This work demonstrates that SlAMADH1 response in the activity assay reflects the aldehyde content of fruit distillates and could be useful for this purpose.

Methods

Chemicals

Aldehydes analyzed as substrates of SlAMADH1 as well as the derivatizing agent DNPH were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). APAL was prepared by a hydrolysis of 3,3-diethoxypropane-1-amine (Sigma–Aldrich Chemie) using hydrochloric acid. Briefly, the diethylacetal (25 μl) was dissolved in 0.4 M HCl to a final concentration of 100 mmol l^{-1} and the solution was incubated at 100°C for 20 min [20]. Methanol for HPLC analysis was obtained from J.T. Baker (Center Valley, PA, USA). All other chemicals were of analytical purity grade. Slivovitz samples were from local non-commercial producers in Moravia, Czech Republic.

Enzyme preparation

SlAMADH1 was produced in *Escherichia coli* as a recombinant protein and purified to homogeneity by liquid chromatography from bacterial lyzate [20,23]. Protein concentration of the final enzyme preparation was determined by the bicinchoninic acid assay [24] with bovine serum albumin as a standard. Results of the protein assay were checked by additional absorption measurements of a diluted enzyme solution at 280 nm (ε_{280} = 75,860 M^{-1} cm^{-1}) [20].

Enzyme kinetics

SlAMADH1 activity assay was based on a spectrophotometrical monitoring of the production of NADH (ε_{340} = 6.3 $mmol^{-1}$ l cm^{-1}) [25] during the enzyme reaction. In a total volume of 2 ml, the reaction mixture contained Tris–HCl buffer (100 mmol l^{-1}, pH 9.0), coenzyme NAD^+ (1 mmol l^{-1}) and 2–10 μl of a stock enzyme solution with a protein concentration of 0.8 mg ml^{-1}. The reaction was initiated by adding substrate (20 μl) into the solution. The concentration of aldehydes in the reaction mixture was 1.0 mmol l^{-1} when the substrate specificity was tested [22], or varied according to the needs for kinetic parameters measurement. In the case of distillates, either 50 μl of a non-concentrated sample or 20 μl of a sample concentrated by evaporation to 2% of the original volume at 45°C on a Concentrator plus (Eppendorf, Hamburg, Germany) were added. All measurements were performed using an Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 30°C. Data analysis was performed with GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA) as described [21]. All kinetic measurements were performed in triplicates.

HPLC analysis coupled with ion-trap MS and MS/MS

Aldehydes in slivovitz samples were detected using reversed-phase HPLC according to a method published for the analysis of spirits made from sugar cane juice [3]. All standard and sample aldehydes were derivatized with DNPH before analysis. Both UV and MS/MS detections were used to monitor the chromatographic separation [13].

Each standard aldehyde (2 mmol) was dissolved in 20 ml of ethanol. DNPH (0.8 g ≈ 4 mmol) was dissolved in 6 ml of water and 4 ml of 96% H_2SO_4. Both solutions were pooled and then shaken at laboratory temperature for one hour. Crystals of DNPHOs were isolated by a filtration and recrystallized from absolute ethanol. Their purity was checked by thin layer chromatography on silica gel plates using dichloromethane as a mobile