Dextrin profiles of starch digested with different amylases

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As a key player in the global yeast production and experts in fermentation processes, Lesaffre designs, manufactures and markets innovative solutions for baking, food taste, health care and biotechnology.

The enzymology group in R&D focuses on enzymes used in baking. Such enzymes can be used to optimize the quality of bread and baking products with regard to volume, texture, shelf-life, color and appearance of crumb and crust. One of the main missions is to identify and characterize different enzymes for designing a bread improver with a balanced combination of baking ingredients that will result in better baking products.

Introduction

Amylases are starch degrading enzymes particularly used for baking. Within the last two years, several new amylases have been commercialized. Despite our deep knowledge of enzyme characterization, we face difficulties in differentiating them by traditional in vitro enzymatic assays (measurement of products formed over time with selected substrates, pH and temperature conditions). Indeed, the activity values of these new amylases were close to each other.

The idea was to assay from the starch substrate the various amylase-produced dextrins ranging from glucose to maltoheptose. The dextrin profile supported understanding of enzyme specificity and effects during baking. HPTLC was a valuable tool to follow several products in parallel. In this specific case, HPTLC did not show any matrix effects and was five times faster compared to the HPLC run of 35 min per sample.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 x 10 cm

Standard solution

The methanolic stock solution mixture (1 mg/mL each) of glucose (DP1), maltose (DP2), maltotriose (DP3), maltotetrose (DP4), maltopentose (DP5), maltohexose (DP6) and maltoheptose (DP7) was diluted 1:20 with methanol.

Sample preparation

The aqueous enzyme solution (1%) was stirred for 20 min, starch solution (4%) was added 1:1 and incubated at 25 °C for 40 min. Heating at 100 °C for 5 min stopped the reaction; if necessary, dilution in methanol.

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 6.0 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volumes between 0.3 and 4.0 µL

Chromatography

In the Automatic Development Chamber (ADC 2) with chamber saturation (with filter paper) and after conditioning for 10 min at 47 % relative humidity using a saturated solution of potassium thiocyanate, development with acetonitrile – acetone – water 3:3:2 up to a migration distance of 60 mm (from the lower edge), drying for 5 min

Postchromatographic derivatization

The plate was immersed into aniline-diphenylamine-phosphoric acid reagent (2 g diphenylamine and 2 mL aniline in 80 mL methanol, 10 mL phosphoric acid, 85%, ad 100 mL methanol) using the Chromatogram Immersion Device (immersion time 1 s and immersion speed 5 cm/s) and heated at 120 °C for 5 min using the TLC Plate Heater

Documentation

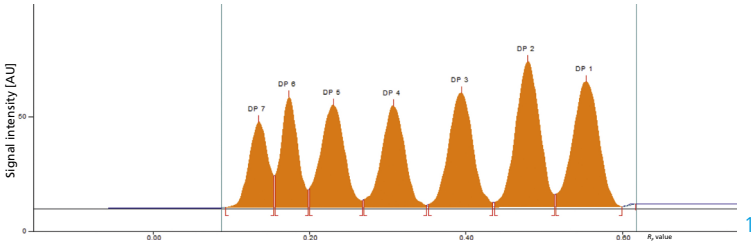
With TLC Visualizer under white light illumination (transmission)

Densitometry

TLC Scanner 4 and winCATS, absorption measurement at 500 nm, slit dimension 4.00 x 0.30 mm, scanning speed 20 mm/s, evaluation via peak area

Results and discussion

The good resolution in the densitogram of the standard mixture DP1 to DP7 allowed a quantification of the formed dextrin products (DPs). The evaluation was done in the polynomial working range (40.0–210.0 ng/band).



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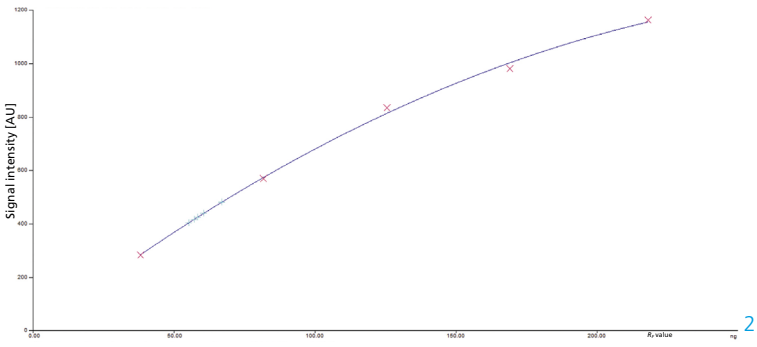
Densitogram of the standard mixture DP1-DP7



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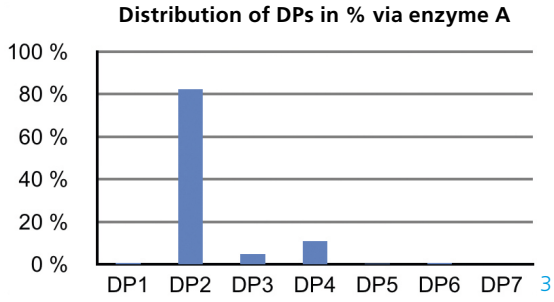
Calibration curve of DP2 as example

After the enzymatic reaction, the product profiles of four different enzyme broths were analyzed. The concentration and percentage of each DP within the product profiles were calculated.

Interestingly, very different DP patterns were observed for the enzymes. For example, with enzyme A mainly DP2 was formed, whereas DP4 was the most abundant product for enzyme B. With enzyme C the major product obtained was DP1. Enzyme D was the best enzyme regarding the distribution among the various DPs with regard to DP1, DP2, DP4 and DP6.



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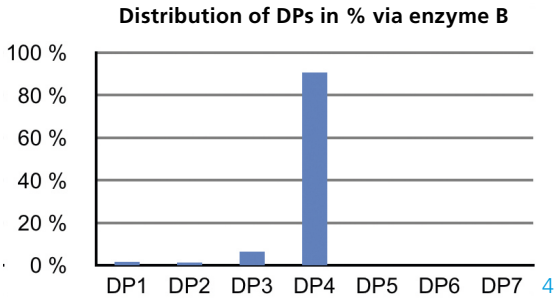

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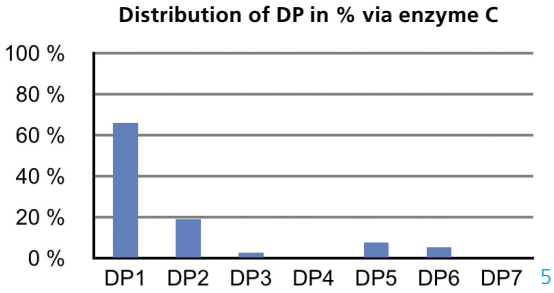

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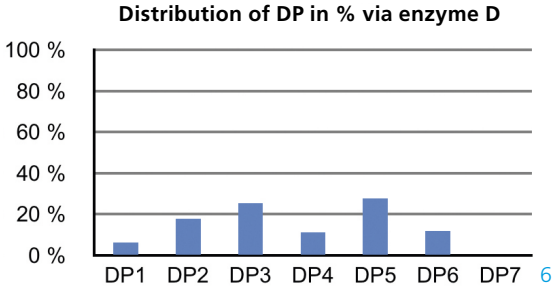

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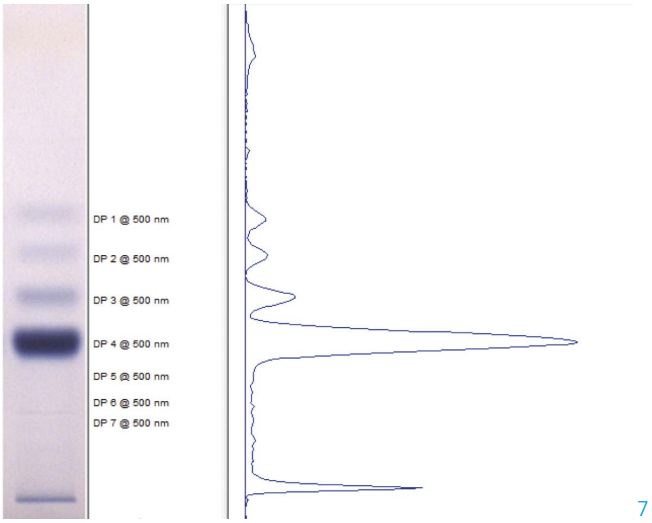

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Chromatogram and densitogram at UV 500 nm of a starch sample digested with enzyme B

A fast and robust method for the quantification of DPs was developed. The method was well suited for the characterization of different enzymes by their formed product profiles. By comparing patterns, enzymes can be differentiated and selected due to their properties. A better understanding of enzyme substrate/product specificity was reached.

Further information is available on request from the authors.

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New: CAMAG TLC Visualizer 2

The professional documentation and evaluation system for Planar Chromatography

The TLC 2 Visualizer is equipped with technical innovations and offers even better image quality than its predecessor:

- Digital CCD camera with a maximum resolution of 82 µm on the plate
- USB 3.0 for easy PC connection
- Simple operation under *visionCATS* (from version 2.3)

The TLC Visualizer 2 is further optimized for the documentation of TLC and HPTLC plates. Through the highest possible homogeneity under all illumination modes – UV 254 nm, UV 366 nm, and white light – ideal and reproducible images are obtained. The software controlled operation offers a broad range of image processing tools and evaluation possibilities, such as a semi-quantitative evaluation of image profiles and a comparison of tracks from different plates with the »Image Comparison«.

The CAMAG TLC Visualizer 2 will be available from October 2016.