New sensitive assay for the quantitative determination of endo- and exo-amylase activities

New assay for the determination of amylase activities with significantly improved sensitivity, lower cost and easier feasibility than comparable assays.

- Significantly increased sensitivity compared to commercial reference products: detection limit of 0.25 pkat mL-1 or 4.5 µM
- Significantly more cost-effective: only 3.4 cents per preparation compared to approx. 1.95 Euro for Ceralpha or 1.93 Euro for Betamyl3
- Arbitrarily scalable due to application in microtiter plates
- Direct application by using natural substrate starch
- Easy to perform at 40° C

Fields of application

Possible applications can be found in all areas where amylases are used. In food industries the assay could be used to determine (residual) amylases in cereal and cereal products, dairy products, and related food products and animal feeds. In the detergent sector amylases crack starchy stains, such as sauces. In textile industry amylases are used to remove the protective starch after the weaving of textiles that are associate with strong mechanical stresses.

Background

Amylases are used in many areas, such as in the food industry, e.g., in the production of baked goods or glucose syrups, the textile industry and the detergent industry. Due to the wide application of amylases, there are many areas in which the sensitive and quantitative determination of amylase activities is of great importance. These include, for example, the determination of amylase activities in wheat flours, as this has a major influence on the quality of baked

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Service
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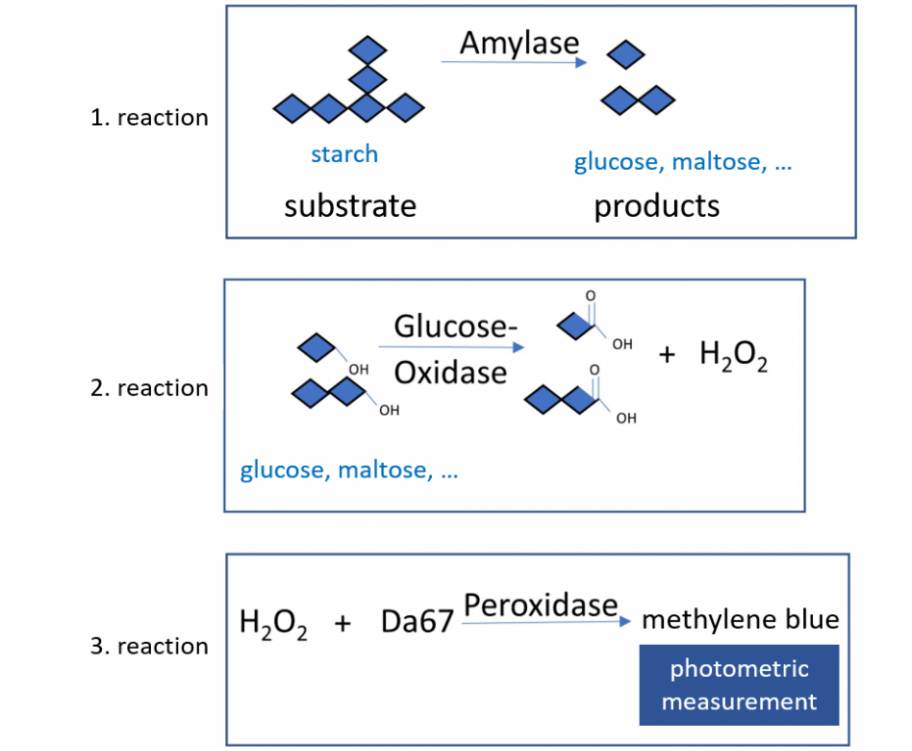
goods. The determination of the residual activities of added amylase preparations in baked bread is also important with regard to a possible declaration obligation.

Problem

Many assays for the determination of amylase activity have already been described in the literature. With the commercially available kits known in the art, amylase activities can be reliably determined up to an enzyme activity of 0.05 U/ml (equivalent to 0.84 nkat/mL) in, for example, foodstuffs. However, the sensitivity of these assays is not sufficient for certain problems, for example for the determination of the residual activity of certain amylases in bread. Thus, the sensitivity of previous assays for the determination of amylase activities is low. Moreover, some of the existing assays involve many and sometimes complex steps (e.g., heating at 95 °C). Further, some known assays use synthetic substrates, which can be disadvantageous for the calculation of required activity amounts.

Solution

Scientists at the University of Hohenheim have developed an improved spectrometric detection test for the determination of endo- and exo-amylase activities in samples. The new detection test is characterized by significantly improved sensitivity, ease of performance, and a significant cost saving compared to other commercial tests. The novel detection method uses two additional enzymes, a glucose oxidase and a peroxidase, as well as the dye methylene blue to detect the natural substrate starch present in the sample under investigation. Comparative studies of the new detection method with available alternatives have shown that the method according to the invention is 4.7 and 4.2 times more sensitive, respectively, than the commercial assays Ceralpha (determination of endo-amylase activity) and Betamyl3 (determination of exo-amylase activity). In contrast to the a forementioned commercial assays, the new detection test directly uses starch as a substrate (and no synthetic substrates), which means that the detection method can be used in various applications close to industry. For example, the method can be used in the food industry to determine amylase activity in wheat flour. At a cost of approximately 3.4 euro cents per assay, the new amylase test is also significantly less expensive than other detection methods.



Sequence of the assay consisting of three individual reactions: Cleavage of long-chain carbohydrates to mono- and disaccharides by amylase, further conversion of mono- and disaccharides by glucose oxidase to hydrogen peroxide and the corresponding acid derivatives. The electron acceptor for this second reaction is the dye Da67, which is reduced by peroxidase to the dye methylene blue. Detection is then carried out by photometric measurements of methylene blue. [Fig.: Professor Dr. L. Fischer, Institut of Biotechnology, University of Stuttgart]