A SCREENING METHODOLOGY FOR THE EVALUATION OF NATIVE STARCH-DEGRADING AMYLASES IN CEREAL-BASED PROCESSING

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Enzymes are increasingly used in industrial processes to tackle challenges like reducing energy consumption, promoting sustainability and enhancing waste valorisation. In cereal-based processes, there is a growing interest in native starch-degrading amylases because of their ability to degrade starch in its native crystalline granular structure, eliminating the need for gelatinisation which typically requires high processing temperatures. Although several native starch-degrading amylases have been identified in the literature, the variety of methods and analytical conditions makes it difficult to compare their effectiveness. We therefore propose a screening methodology to evaluate the potential of native starch-degrading amylases under relevant conditions.

Nine promising native starch-degrading amylases were selected after an extensive literature review for a comparative study. These enzymes, all endo-amylases, originated from both bacterial and fungal sources. The first element of the screening methodology was to monitor starch degradation over time by measuring the production of reducing sugars. As the scope was set on cereal-based processes, the screening was performed at pH 5.0 and 40°C. Reducing sugar concentrations were determined by a colourimetric assay, namely the BCA assay. With this approach, different native starch-degrading amylases with potential for cereal-based processing conditions could be identified. Complete starch degradation was defined as the amount of reducing sugars produced when starch is fully hydrolysed to glucose. Starch degradation levels between 15% and 44% were measured after an incubation of 24h with 0.08 Ceralpha Units amylase (as defined with the Ceralpha assay from Megazyme, Bray, Ireland). By measuring starch degradation at multiple time points, a kinetic study was performed to determine the degradation rates, which ranged from 0.05/s to 0.94/s.

The second element of the screening methodology was to assess the capacity of the amylases to bind to native starch. The binding capacity ranged from 0% to 70% with 100% binding capacity indicating complete binding of all amylases to the native starch granules. Strong differences were observed between the selected amylases. The results showed that amylases with higher binding capacity tended to show higher degradation rates and/or higher native starch degradation levels. This binding assay could thus provide initial insights into the capabilities of the amylases to degrade native starch, without the need for extensive experimental work. The two elements of the screening methodology allowed us to compare the ability of the selected amylases to degrade native starch in the context of cereal-based processes.