High-pressure applications for enzyme conversions in the food industry

The use of elevated temperature and pressure can be highly advantageous for controlling enzyme reactions. Unlike temperature, pressure is usually neglected as a fundamental process parameter. Besides the availability of the high-pressure equipment, also enzyme instability at elevated pressures is a reason. Therefore, studies on high-pressure enzymatic reactions have focussed on the behaviour of the structure of the enzyme and its (un)folding under pressure. Less attention has been given to functionality. In our work, we have focussed on this functionality and during the presentation high pressure in enzymatic starch hydrolysis (gelatinization of starch), in protein hydrolysis and oligosaccharide synthesis will be discussed.

Enzymatic reactions can be manipulated by pressure. There are two thermodynamic effects of pressure. First, kinetic effects on the reaction rates, controlled by the activation volume, and second, effects on the reaction equilibrium, controlled by changes in reaction volume before and after reaction. These effects are comparable with temperature effects due to the Gibbs (activation) energy. However, the nature of the pressure effects is completely different and follows different rules than the dependence on temperature.
Beside this possible positive effect on the speed and equilibrium of a reaction, the application of high pressure can also affect reaction selectivity, product and substrate solubility, and different phase transport phenomena can occur when solvents are used.

Oligosaccharide synthesis
High-pressure experiments with the hyperthermophilic β-glycosidase from *Pyrococcus furiosus* were performed at our laboratory. Oligosaccharides were synthesized from glucose in an equilibrium reaction at pressures from 0.1 to 500 MPa. The enzyme remained active at 500 MPa. The equilibrium of the reaction was influenced by pressure and shifted towards the hydrolysis side, decreasing the final oligosaccharide concentrations with increasing pressure.

Protein hydrolysis
High pressure has also be applied to the enzymatic hydrolysis of β-Casein. β-Casein is present in the form of micelles at atmospheric pressure. High pressure treatment during the hydrolysis improves the accessibility of the protein. Two proteolytic enzymes with a distinct different function were used on the β-casein during pressurization. Trypsin that mainly hydrolyses the hydrophilic part of the β-casein and chymotrypsin that hydrolyses hydrophobic peptide bounds. Peptide profiles were measured via HPLC. For comparison, the enzymatic hydrolysis was also measured in time at atmospheric conditions.
Tryptic activity was reduced under pressure, but pressure did not influence the reaction mechanism, probably because the hydrophilic part of β-casein is sufficiently accessible. However, chymotryptic proteolysis under pressure yielded different, new peptides under pressure that could not be explained by a change in enzyme activity. Therefore, in this case, pressure altered the mechanism of hydrolysis, which led to different peptides that can have different properties.

Starch gelatinization
A third example of the application of high pressure to enzyme conversions is the gelatinization of starch prior to the enzymatic hydrolysis. Starch can be gelatinized by means of a temperature increase or by means of a pressure increase. We have studied the effect of temperature, pressure and treatment time on the degree of gelatinization. These results were obtained with differential scanning calorimetry measurements for wheat starch-water mixtures with starch concentrations varying between 5 and 80 w/w %. A thermodynamic model based on the Gibbs energy difference was used to describe the degree of gelatinization as a function of both pressure and temperature. The experimental and model data were used to construct a phase diagram for 5, 30, and 60 w/w % wheat starch-water mixtures. These phase diagrams can be used to estimate the

degree of gelatinization after applying a certain pressure and temperature on a starch-water mixture with starch concentrations in the range of 5 and 60 w/w %. We also made a comparison between the effect of both gelatinization methods on the composition and reaction rate during enzymatic hydrolysis of wheat starch. We found that the product composition was affected by the type of starch pretreatment and the enzyme addition point, but it was not affected by the hydrolysis pressure, as long as gelatinization was complete. The differences between thermally gelatinized, high pressure gelatinized and native starch were explained by considering the accessibility of starch during the hydrolysis. These results showed that the product composition could be influenced by choosing different process sequences and conditions.

To summarize, this presentation shows that pressure is an interesting parameter to use for enzyme conversions. In future, it may be necessary to isolate enzymes with a better pressure stability and to design reactor concepts that can reliably and cost-effectively apply these high pressures.

Literature:

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