

# Thiolase

## Sulphydryl oxidase – a new flour enzyme cross-links proteins

### Background

The suggestion for using sulphydryl oxidase (SOX, EC 1.8.3.2) derived from *Aspergillus sojae* in baked goods and dairy products was first made some twenty years ago. SOX was never produced on an industrial scale, the reason being that it was thought to inhibit the organisms from which it was derived; moreover, the enzyme was unstable.

Using the methods of modern biotechnology a research team made up from universities, startups and SternEnzym has succeeded in modifying the genetic code of SOX from *S. cerevisiae* (baker's yeast) and transferring it to *B. subtilis*. In this way we have met the most important requirement for industrial-scale production. We are currently optimizing the process with the aim of making it more economical.

### What is thiolase?

**Thiolase** is a pure sulphydryl oxidase. It belongs to the oxidoreductase class (EC 1.8.3.2) and catalyzes the oxidation of two sulphydryl groups (thiol groups) to create a disulphide bridge, forming hydrogen peroxide in the process. The scheme is as follows:



Although the enzyme is derived from baker's yeast, the functions described below can only be achieved with the isolated enzyme, not with yeast cells.

- The main task of **thiolase** is to oxidize the sulphydryl groups.

### The effects of thiolase in flour

The stable structure of bread depends to a large extent on the proteins present in the flour; these consist of long chains of amino acids. One of these amino acids (cysteine) contains a reactive sulphydryl group which can cross-link with other sulphydryl groups. During proving and baking a highly specific form of oxidation takes place under the influence of the sulphydryl oxidase:

- The sulphydryl groups join up in pairs to form disulphide bridges. This strengthens the proteins and thus the dough.

- Via the disulphide bridges the protein molecules can link up with themselves (intramolecularly) or with one or more other protein molecules (intermolecularly) to form a network of chemically bonded protein.
- The rheological properties of a dough result from this network.

### Sulphydryl oxidase (SOX) versus glucose oxidase (GOX)

Because of its small size and the chemically stable bonding of its co-enzyme FAD, **thiolase** is much more resistant to heat and oxygen than glucose oxidase (the oxidase most often used for flour at present).



Comparison of glucose oxidase and sulphydryl oxidase in steamed bread.

Glucose oxidase is produced by *Aspergillus* or *Penicillium* species and consists of 2 or 4 large sub-units. The co-enzyme FAD is only loosely linked to the protein and is therefore easily lost during storage or processing. **Thiolase** has been given extra stability through the patented exchange of amino acids.

### Use of thiolase in bakery products

**Thiolase** is a protein crosslinker. It has shown itself to be an ideal synergist with other flour improvers such as enzymes, reducing or oxidizing agents and also emulsifiers. Tests have shown that the use of **thiolase** can reduce the amount of flour maturing agents normally required.

With regard to dough rheology, **thiolase** is even effective in spite of limited time in the Farinograph and Alveograph, where it increases the stability or resistance of the dough.

It has not yet been possible to investigate all applications systematically. Very good results have so far been achieved in the following:

### Laminated dough

For example Chinese steamed buns, puff pastry products, Danish pastries, baklava-type products and pasties



### Croissants

Especially in the production of frozen dough pieces and split rolls



### Baking premixes/baking improvers

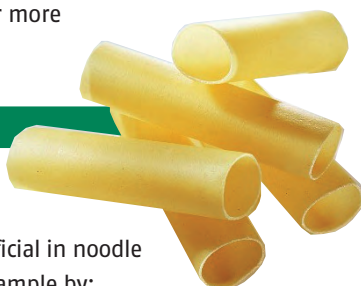
Synergistic effects have been shown to occur in mixtures of baking-active substances, making it possible to reduce the amount of other oxidizing agents added. Unlike the oxidizing agents currently in general use, **thiolase** acts slowly; the best stability of the dough is therefore reached at the end of the proof time, the doughs are pleasant to handle and do not become "bucky" through over-oxidation.

- Larger volume
- Good stability and fermentation tolerance of the dough portions
- Stabilization of the finished bakery products
- In contrast to glucose oxidase, no effect on odour or flavour and no rancidity in products containing butter, even if these are stored for more than 10 weeks.

### Noodles and pasta

On the basis of our experience with bakery products we expect thiolase to prove beneficial in noodle and pasta applications, for example by:

- making it possible to reduce the proportion of hard wheat
- improving tolerance to boiling.



### Specification

Sulphydryl oxidase (method: 100 mu/g ( <b>thiolase 100</b> ) DTT, 30 °C, pH 7.4, 412 nm]	
Appearance:	Beige or yellowish powder
Odour:	Typical, fermentation
Typical dosage: (baking applications)	100–250 ppm (flour basis)

### Temperature and pH curves

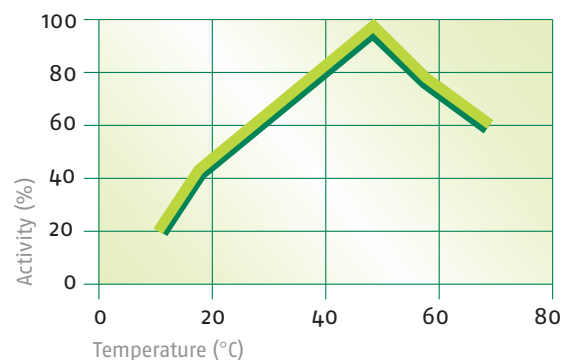


Fig. 1: Temperature-activity curve for **thiolase**

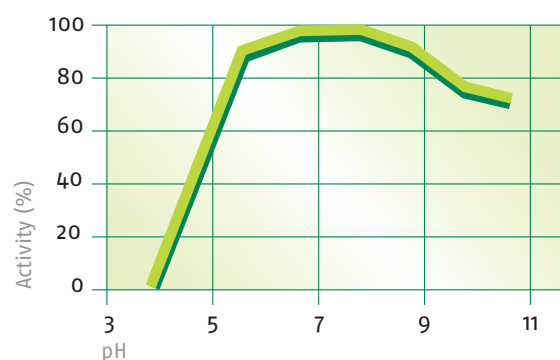


Fig. 2: pH-activity curve for **thiolase**

Optimum temperature range: 30–60°C  
Optimum pH range: 5.5–9