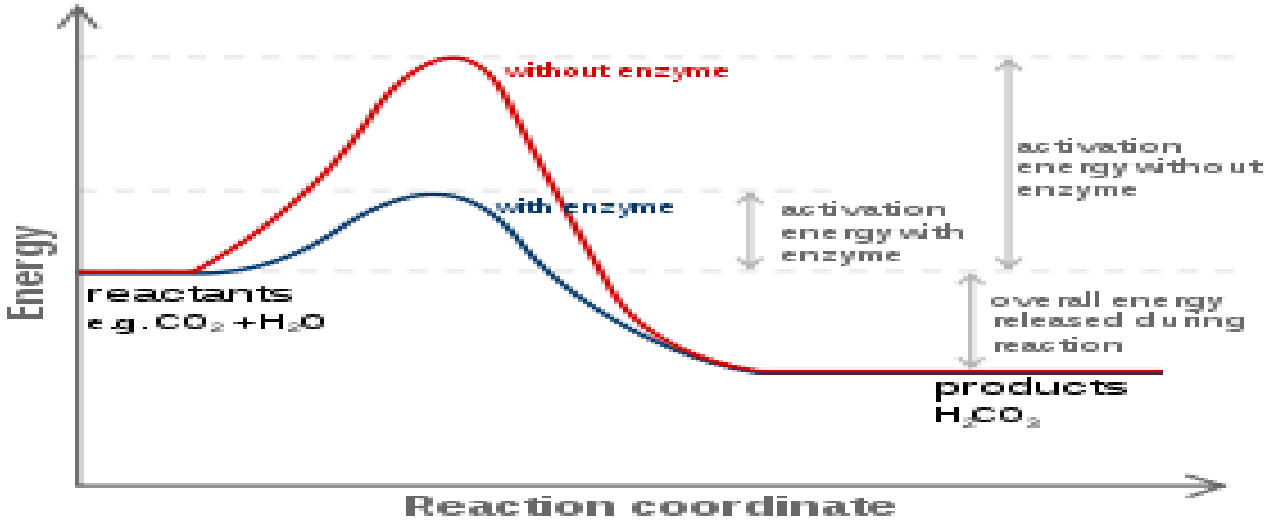
العوامل المؤثرة على التفاعلات الانزيمية

Factors affecting Enzyme-Catalyzed Reactions



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Factors affecting Enzyme-Catalyzed Reactions

1- Effect of Substrate Concentration (Single-Substrate Reactions)

2- Effect of Inhibitors

3- Effect of pH

4- Effect of Temperature

5- Effect of Pressure

6- Effect of Water

-Enzymes in food can be detected only indirectly by measuring their catalytic activity and, in this way, differentiated from other enzymes.

-This is the rationale for acquiring knowledge needed to analyze the parameters which influence or determine the rate of an enzyme-catalyzed reaction.

-The reaction rate is dependent on the concentrations of the components involved in the reaction.

-Here, it means primarily the substrate and the enzyme. Also, the reaction can be influenced by the presence of activators and inhibitors.

Finally, the pH, the ionic strength of the reaction medium, the dielectric constant of the solvent (usually water) and the temperature exert an effect.

1 Effect of Substrate Concentration
Single-Substrate Reactions

-Let us consider a single-substrate reaction.

-Enzyme E reacts with substrate A to form an intermediary enzyme-substrate complex, EA.
The complex then forms the product P and releases the free enzyme:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \xrightarrow{k_2} E + P$$

-In order to determine the catalytic activity of the enzyme, the decrease in substrate concentration or the increase in product concentration as a function of time can be measured.

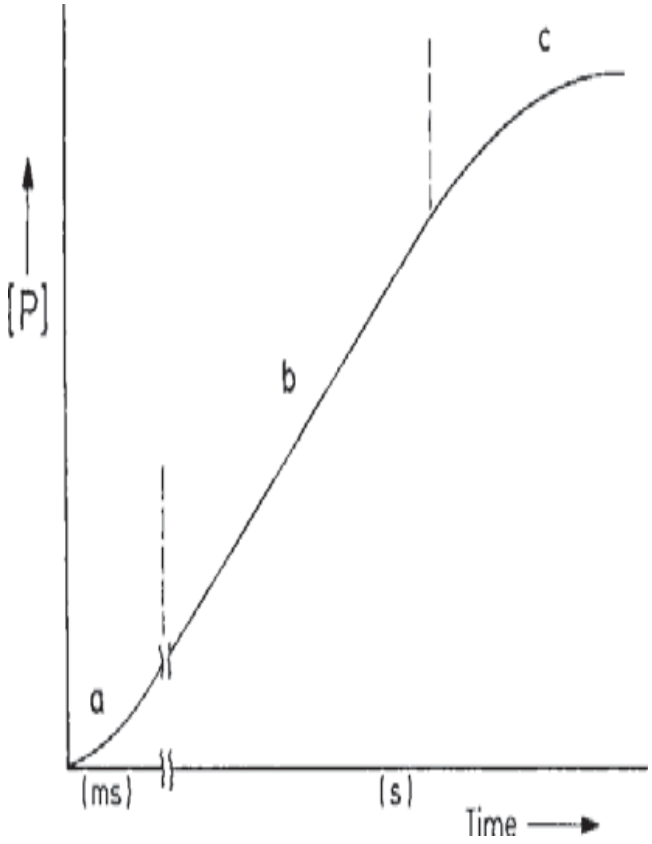
1 Effect of Substrate Concentration
Single-Substrate Reactions

The activity curve obtained has the following regions:

a) The maximum activity which occurs for a few msec until an equilibrium is reached between the rate of enzyme-substrate formation and rate of breakdown of this complex.

-Measurements in this pre-steady state region which provide an insight into the reaction steps and mechanism of catalysis are difficult and time consuming.

-Hence, further analysis of the pre-steady state should be ignored.



Progress of an enzyme-catalyzed reaction

b) The usual procedure is to measure the **enzyme activity** when a **steady state** has been reached. In the **steady state**, the intermediary **complex concentration** remains constant while the **concentration of the substrate and end product are changing**.

C) The reaction rate **decreases** in this region in spite of an excess of substrate.

The decrease in the reaction rate can be considered to be result of:

-**Enzyme denaturation** which can readily occur,

-**Decreasing the enzyme concentration** in the reaction system, or the product formed **increasingly inhibits enzyme activity** or, after the concentration of the product increases,

-**The reverse reaction takes place**, converting the product back into the initial reactant.

2 Effect of Inhibitors

-The catalytic activity of enzymes, in addition to substrate concentration, is affected by the type and concentration of inhibitors, i.e. compounds which decrease the rate of catalysis, and activators, which have the opposite effect.

-Activitors include
-metal ions and
-compounds which are active as prosthetic groups or which provide stabilization of the enzyme's conformation or of the enzyme-substrate complex.

-Inhibitors are found among food constituents.

2 Effect of Inhibitors

-Based on kinetic considerations, inhibitors are divided into two groups:
inhibitors bound *irreversibly* to enzyme and
inhibitors bound *reversibly* to enzyme .

-Proteins which specifically inhibit the activity of certain peptidases, amylases or β-fructofuranosidase are examples.

-Furthermore, food contains substances which non-selectively inhibit a wide spectrum of enzymes.

-Phenolic compounds of food and mustard oil belong to this group.

2 Effect of Inhibitors

-Food might be contaminated with pesticides, heavy metal ions and other chemicals from a polluted environment which can become inhibitors under some circumstances.

-These possibilities should be taken into account when enzymatic food analysis is performed.

-Food is usually heat treated to suppress undesired enzymatic reactions.

-As a rule, no inhibitors are used in food processing.

-An exception is the addition of, for example, SO_2 to inhibit the activity of phenolase.

2 Effect of Inhibitors

-Much data concerning the mechanism of action of enzyme inhibitors have been published in recent biochemical research.

These data cover

-the elucidation of the effect of inhibitors on functional groups of an enzyme,

-the effect of inhibitors on the active site and

-the clarification of the general mechanism involved in an enzyme catalyzed reaction.

3 Effect of pH on Enzyme Activity

-Each enzyme is active only in a narrow pH range and each has a pH optimum which is often between pH 5.5 and 7.5 (Table).

-The optimum pH is affected by the type and ionic strength of the buffer used in the assay.

pH Optima of various enzymes

Enzyme	Source	Substrate	pH Optimum
Pepsin	Stomach	Protein	2
Chymotrypsin	Pancreas	Protein	7.8
Papain	Tropical plants	Protein	7–8
Lipase	Microorganisms	Olive oil	5–8
α-Glucosidase (maltase)	Microorganisms	Maltose	6.6
β-Amylase	Malt	Starch	5.2
β-Fructofuranosidase (invertase)	Tomato	Saccharose	4.5
Pectin lyase	Microorganisms	Pectic acid	9.0–9.2
Xanthine oxidase	Milk	Xanthine	8.3
Lipoxygenase, type I[a]	Soybean	Linoleic acid	9.0
Lipoxygenase, type II[a]	Soybean	Linoleic acid	6.5

3 Effect of pH on Enzyme Activity

The reasons for the sensitivity of the enzyme to changes in pH are:

a) Sensitivity is associated with a change in protein structure leading to irreversible denaturation,

b) The catalytic activity depends on the quantity of electrostatic charges on the enzyme's active site generated by the prototropic groups of the enzyme.

-In addition the ionization of dissociable substrates as affected by pH can be of importance to the reaction rate.

-However, such effects should be determined separately.

4 Influence of Temperature

-Thermal processes are important factors in the processing and storage of food because they allow the control of chemical, enzymatic and microbial changes.

-Undesired changes can be delayed or stopped by refrigerated storage. Heat treatment may either accelerate desirable chemical or enzymatic reactions or inhibit undesirable changes by inactivation of enzymes or microorganisms.

-Temperature and time are two parameters responsible for the effects of a thermal treatment.

-They should be selected carefully to make sure that all necessary changes, e. g., killing of pathogens, are guaranteed, but still all undesired changes such as degradation of vitamins are kept as low as possible.

-The following table informs about quality deterioration caused by enzymes which can be eliminated e. g., by thermal inactivation.

Thermal inactivation of enzymes to prevent deterioration of food quality

Food product	Enzyme	Quality loss
Potato products, apple products	Monophenol oxidase	Enzymatic browning
Semi-ripe peas	Lipoxygenase, peroxidase	Flavor defects; bleaching
Fish products	Proteinase, thiaminase	Texture (liquefaction), loss of vitamine B_1
Tomato purée	Polygalacturonase	Texture (liquefaction)
Apricot products	β-Glucosidase	Color defects
Oat flakes	Lipase, lipoxygenase	Flavor defects (bitter taste)
Broccoli Cauliflower	Cystathionine β-Lyase (cystine-lyase)	Off-flavor

4 Influence of Temperature

Temperature Optimum

-Enzyme-catalyzed reactions and the growth of microorganisms show a so-called temperature optimum, which is a temperature-dependent maximum resulting from the overlapping of two counter effects with significantly different activation energies:

- increase in reaction or growth rate
- increase in inactivation or killing rate

4 Influence of Temperature

Temperature Optimum

-For starch hydrolysis by microbial α-amylase, the activation energies were :

- Ea (hydrolysis) = 20 kJ · mol^{-1}
- Ea (inactivation) = 295 kJ · mol^{-1}

-As a consequence of the difference in activation energies, the rate of enzyme inactivation is faster with increasing temperature than the rate of enzyme catalysis.

-Based on activation energies for the above example, the following relative rates are obtained (Table).

-Increasing temperature from 0 to 60 ◦C increases the hydrolysis rate by a factor of 5, while rate of inactivation is accelerated by more than 10 powers of ten.

Temperature Optimum

α-Amylase activity as affected by temperature: relative rates of hydrolysis and enzyme inactivation

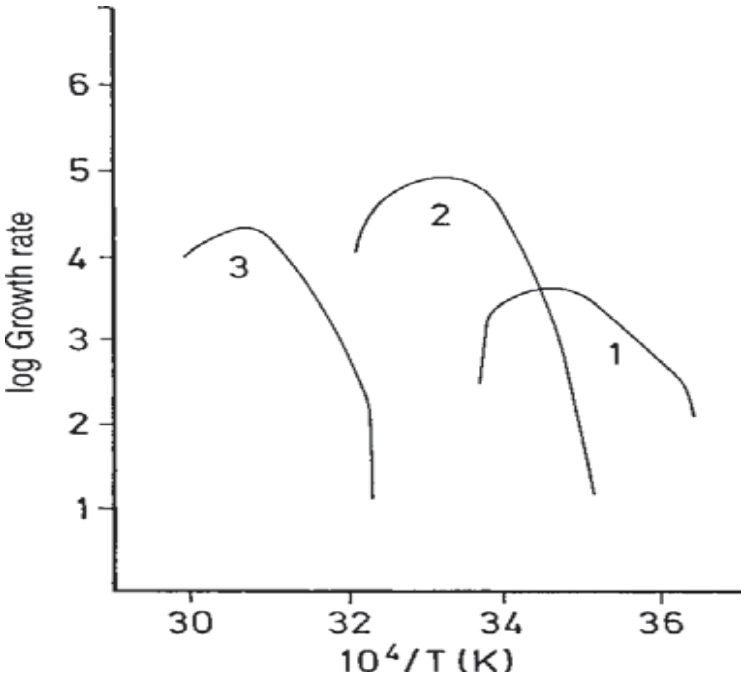
Temperature (°C)	Relative rate[a]	
	hydrolysis	inactivation
0	1.0	1.0
10	1.35	$1.0 \cdot 10^2$
20	1.8	$0.7 \cdot 10^4$
40	3.0	$1.8 \cdot 10^7$
60	4.8	$1.5 \cdot 10^{10}$

[a] Activation energies of 20 kJ · mole^{-1} for hydrolysis and 295 kJ · mole^{-1} for enzyme inactivation were used for calculation according to *Whitaker* (1972).

Temperature Optimum

-The growth of microorganisms follows a similar temperature dependence and can also be characterized by replacing the value k by the growth rate and assuming Ea is the reference value μ of the temperature for growth (Figure).

-For maintaining food quality, detailed knowledge of the relationship between microbial growth rate and temperature is important for optimum production processes (heating, cooling, freezing).



Growth rate and temperature for 1) psychrophilic (*Vibrio AF-1*), 2) mesophilic (*E. coli*) and 3) thermophilic (*Bacillus cereus*) microorganisms

Temperature Optimum

-The highly differing activation energies for killing microorganisms and for normal chemical reactions have triggered a trend in food technology towards the use of high-temperature short-time (HTST) processes in production.

-These are based on the findings that at higher temperatures the desired killing rate of microorganisms is higher than the occurrence of undesired chemical reactions.

Thermal Stability

-The thermal stability of enzymes is quite variable.

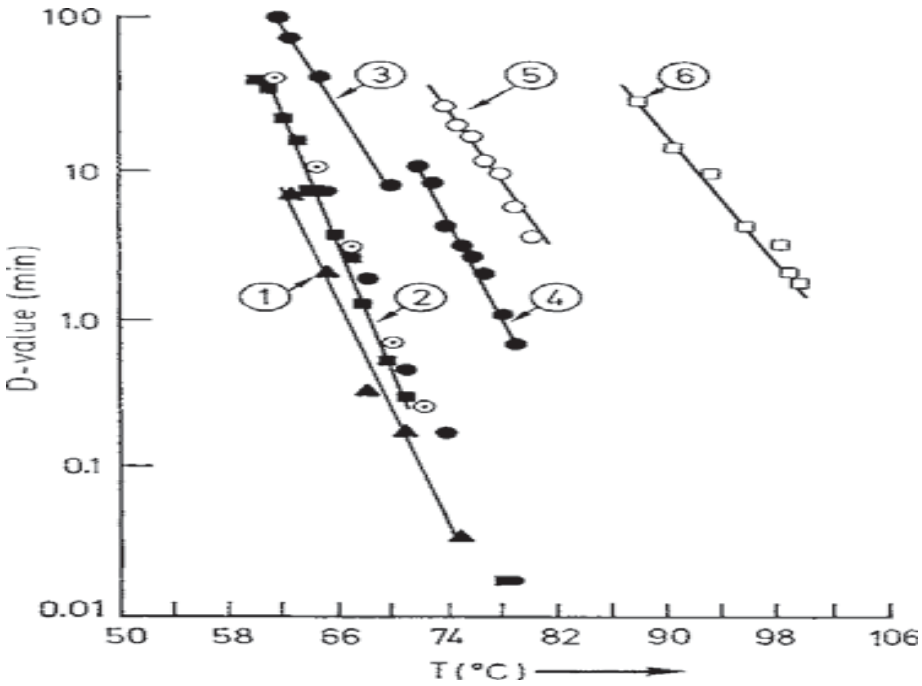
-Some enzymes lose their catalytic activity at lower temperatures, while others are capable of withstanding (at least for a short period of time) a stronger thermal treatment.

-In a few cases enzyme stability is lower at low temperatures than in the medium temperature range.

Thermal Stability

-Lipase and alkaline phosphatase in milk are thermolabile, whereas acid phosphatase is relatively stable (Figure).

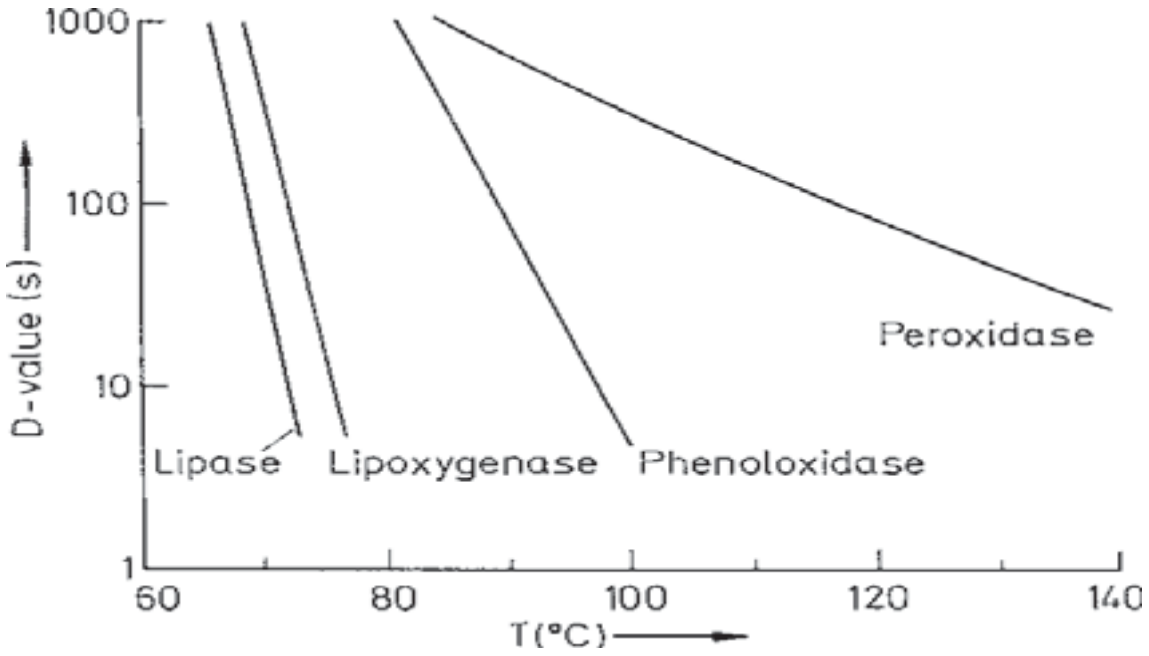
-Therefore, alkaline phosphatase is used to distinguish raw from pasteurized milk because its activity is easier to determine than that of lipase.



Thermal inactivation of enzymes of milk.
1 Lipase (inactivation extent, 90%),
2 alkaline phosphatase (90%),
3 catalase (80%),
4 xanthine oxidase (90%),
5 peroxidase (90%), and
6 acid phosphatase (99%)

Thermal Stability

-Of all the enzymes in the potato tuber (Figure), peroxidase is the last one to be thermally inactivated.



Thermal inactivation (90%) of enzymes present in potato tuber

Thermal Stability

-Such inactivation patterns are often found among enzymes in vegetables. In such cases, peroxidase is a suitable indicator for controlling the total inactivation of all the enzymes e.g., in assessing the adequacy of a blanching process.

-However, newer developments aim to limit the enzyme inactivation to such enzymes responsible for quality deterioration during storage.

-For example pea seeds in which lipoxygenase is responsible for spoilage. However, lipoxygenase is more sensitive than peroxidase, thus a sufficient but gentle blanching requires the inactivation of lipoxygenase only. Inactivation of peroxidase is not necessary.

5 Influence of Pressure

-The application of high pressures can inhibit the growth of microorganisms and the activity of enzymes. This allows the protection of sensitive nutrients and aroma substances in foods.

-Some products preserved in this gentle way are now in the market.

-Microorganisms are relatively sensitive to high pressure because their growth is inhibited at pressures of 300–600 MPa and lower pH values increase this effect. However, bacterial spores withstand pressures of >1200 MPa.

-In contrast to thermal treatment, high pressure does not attack the primary structure of proteins at room temperature. Only H-bridges, ionic bonds and hydrophobic interactions are disrupted. Quaternary structures are dissociated into subunits by comparatively low pressures (<150 MPa).

5 Influence of Pressure

-Higher pressures (>1200 MPa) change the tertiary structure and very high pressures disrupt the H-bridges which stabilize the secondary structure.

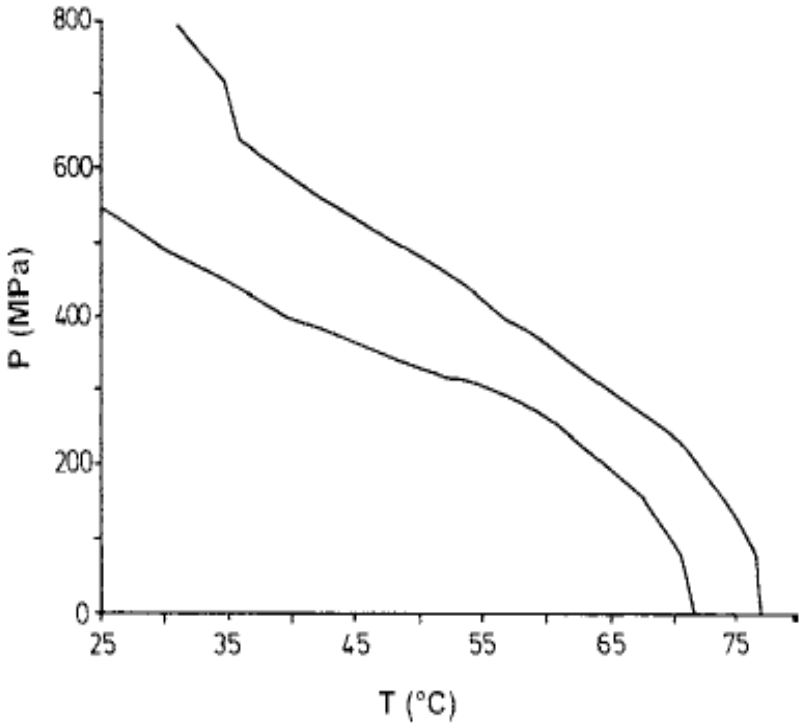
-The hydration of proteins is also changed by high pressure because water molecules are pressed into cavities which can exist in the hydrophobic interior of proteins.

-In general, proteins are irreversibly denatured at room temperature by the application of pressures above 300 MPa while lower pressures cause only reversible changes in the protein structure.

5 Influence of Pressure

-In the case of enzymes, even slight changes in the steric arrangement and mobility of the amino acid residues which participate in catalysis can lead to loss of activity.

-Nevertheless, a relatively high pressure is often required to inhibit enzymes. But the pressure required can be reduced by increasing the temperature, as shown in the Figure for α-amylase.



Pressure-temperature diagram for the inactivation kinetics of α-amylase from *Bacillus subtilis at* pH *8.6.*

5 Influence of Pressure

-While a pressure of 550 MPa is required at 25 ◦C to inactivate the enzyme, a pressure of only 340 MPa is required at 50 ◦C.

-It is remarkable that enzymes can also be activated by changes in the conformation of the polypeptide chain, which are initiated especially by low pressures around 100 MPa.

-In the application of the pressure technique for the production of stable food, intact tissue, and not isolated enzymes, is exposed to high pressures.

-Thus, the enzyme activity can increase instead of decreasing when cells or membranes are disintegrated with the release of enzyme and/or substrate.

5 Influence of Pressure

-Some examples are presented here to show the pressures required to inhibit the enzyme activity which can negatively effect the quality of foods.

–Pectin methylesterase: causes the flocculation of pectic acid in orange juices and reduces the consistency of tomato products. In orange juice, irreversible enzyme inactivation reaches 90% at a pressure of 600 MPa. Even though the enzyme in tomatoes is more stable, increasing the temperature to 59–60 ◦C causes inactivation at 400 Mpa and at 100 MPa after the removal of Ca^{2+} ions.

–Peroxidases: induce undesirable aroma changes in plant foods. In green beans, enzyme inactivation reached 88% in 10 min after pressure treatment at 900 MPa. At pressures above 400 MPa (32 ◦ C), the activity of this enzyme in oranges fell continuously to 50%. However, very high pressures increased the activity at 32–60 ◦C.

5 Influence of Pressure

-It is possible that high pressure denatures peroxidase to a heme(in) catalyst to 50%. However, very high pressures increased the activity at 32–60 ◦C. It is possible that high pressure denatures peroxidase to a heme(in) catalyst.

– Lipoxygenase from soybeans.

This enzyme was inactivated in 5 min at pH 8.3 by pressures up to 750 MPa and temperatures in the range 0–75 ◦C. The pressure stability was reduced by gassing with CO_2 and reducing pH to 5.4.

–Polyphenol oxidases in mushrooms and potatoes require pressures of 800–900 Mpa for inactivation. The addition of glutathione (5 mmol/l) increases the pressure sensitivity of the mushroom enzyme. In this case, the inactivation is obviously supported by the reduction of disulfide bonds.

6 Influence of Water

-Up to certain extent, enzymes need to be hydrated in order to develop activity.

-Hydration of e.g. lysozyme was determined by IR and NMR spectroscopy.

-As can be seen in Table, first the charged polar groups of the side chains hydrate, followed by the uncharged ones.

Hydration of Lysozyme

g Water / g Protein	Hydration sequence	Molecular changes
0.0	Charged groups	Relocation of protons
	Uncharged, polar groups (formation of clusters)	New orientation of disulfide bonds
0.1	Saturation of COOH groups Saturation of polar groups in side chains	Change in conformation
0.2	Peptide-NH	Start of enzymatic activity
0.3	Peptide-CO Monomolecular hydration of polar groups Apolar side chains	
0.4	Complete enzyme hydration	

6 Influence of Water

-Enzymatic activity starts at a water content of 0.2 g/g protein, which means even before a monomolecular layer of the polar groups with water has taken place.

-Increase in hydration resulting in a monomolecular layer of the whole available enzyme surface at 0.4 g/g protein raises the activity to a limiting value reached at a water content of 0.9 g/g protein. Here the diffusion of the substrate to the enzyme's active site seems to be completely guaranteed.

-For preservation of food it is mandatory to inhibit enzymatic activity completely if the storage temperature is below the phase transition temperature.