

**Novel Technologies for Food Processing and Shelf Life Extension**  
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**Lecture – 11**  
**High Pressure Processing of Food (Part – 1)**

High pressure processing of foods is organized in two parts. In part 1, the process principle and technological aspects of high pressure processing is discussed, whereas in part 2, the effect of high pressure process parameters on food constituents and its quality is included.

**High pressure processing (HPP)**

- HPP is a **novel** method for non thermal processing of food.
- It, a relatively **new concept** compared to conventional thermal processing, is receiving wide attention.
- HPP is also termed as
  - ✓ Hyperbaric pressure
  - ✓ Ultra high pressure
  - ✓ High hydrostatic pressure
  - ✓ Pascalization
- In HPP, the food is subjected to elevated pressures (upto **900 MPa or 9000 atm**) with or without the addition of heat to achieve microbial inactivation or to alter the food attributes in order to achieve desired qualities.

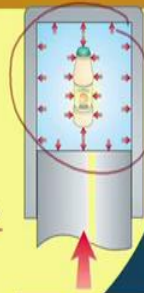

The slide includes two images: a green bear-shaped pressure vessel labeled '5000 kg' and a photograph of a large industrial HPP processing line. At the bottom right, there is a video inset of Prof. Hari Niwas Mishra speaking. The bottom of the slide features logos for 'swayam' and other educational institutions.

High pressure processing (HPP) is a novel method for non-thermal food processing. It is receiving worldwide attention now and is a new concept as compared to the conventional thermal processes. High pressure processing is also termed as hyperbaric pressure, ultra high pressure, high hydrostatic pressure processing or pascalization, etc.

In HPP, the food is subjected to elevated pressure (up to 900 MPa or 9000 atm) with or without the addition of heat to achieve microbial inactivation, enzymatic inactivation or to alter the food attributes in order to achieve desired qualities.

**Process principles**

- **Iso-static principle**  
Application of pressure is instantaneous and uniform through out the sample.
- **Le Chatelier's Principle**  
Reaction volume change is influenced by high pressure applications. Reactions resulting in a volume decrease are accelerated with the application of HP and vice-versa.
- **Principle of microscopic reordering**  
At constant temperature increase in pressure increases the degree of ordering of molecules. Therefore, pressure and temperature exert antagonistic forces on molecular structure & chemical reaction.

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## Process Principles

There are basically three principles involved in high pressure processing –

### (i) Iso-static principle

It states that application of pressure is instantaneous and uniform throughout the sample

### (ii) Le Chatelier's principles

It states that the reaction volume change is influenced by high pressure applications during high pressure processing and this change result into the volume decrease which is accelerated with the application of high pressure and vice versa.

### (iii) Principle of microscopic reordering

It states that at a constant temperature, increase in pressure increases the degree of ordering of molecules. Therefore, pressure and temperature exert antagonistic forces and molecular structure and chemical reactions.

### HPP Technology

Package food in a sterilized container  
 Load packed food in a pressure chamber  
 Fill the pressure chamber with water  
 Pressurize the chamber  
 Hold under pressure  
 De-pressurize the chamber  
 Remove processed food

100-900MPa

## HPP Technology

As far as the technology of high pressure processing is concerned, normally the food material is taken in a sterilized package/container. This packaged food material is loaded in the pressure chamber of HPP equipment and then the desired pressure is applied. The food material is held at the desired pressure for desired duration of time. At the end, the pressure is released to depressurize the chamber and the package is removed.

The schematic diagram of HPP equipment is shown (see Fig.) which consist of pressure chamber/vessel with the arrangements for heating, cooling to hold the product and suitable type of pressure pump to generate high pressure. The pressure vessel is completely or properly sealed.

### Pressure generation system

Direct compression

Indirect compression

## Pressure generation system

There are two types of pressure generation system which are used in the commercial equipment of the high pressure processing (i) Direct compression system (ii) Indirect compression system.

In the case of direct compression system (see Fig.), the compression fluid (normally water) is loaded into the pressure chamber and then pressure vessel is closed at the bottom and top. In direct compression system, a pressure vessel is provided with the piston arrangement. The medium i.e. water inside the vessel is pressurized by piston with the help of a low pressure pump to develop the desired pressure.

In case of indirect compression systems, the fluid is pressurized somewhere else i.e. in pressure medium tank and then this pressurized fluid is loaded in the pressure chamber/vessel of the HPP equipment through or with the help of an intensifier.

**Components of HPP System**

- A pressure vessel
- Closure(s) for sealing the vessel
- A device for holding the closure(s) in place while the vessel is under pressure (e.g., yoke)
- High-pressure intensifier pump(s)
- Pressure & temperature (optional) controlling and monitoring system
- A product-handling system

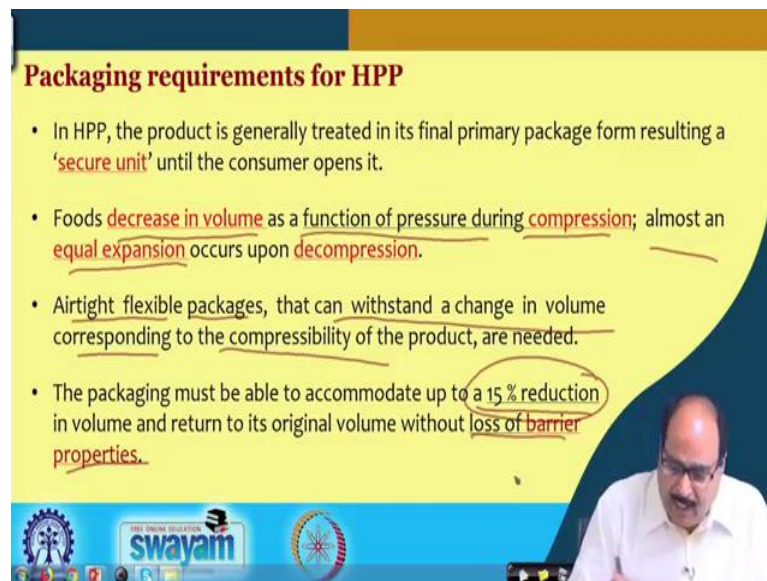
The slide includes a photograph of a high-pressure vessel on the left, a technical diagram of a piston-cylinder arrangement at the top right, and a detailed diagram of the vessel's closure mechanism at the bottom right. A small inset photo of a man speaking is located in the bottom right corner. The slide also features the Swayam logo and other icons at the bottom.

## Components of HPP system

The high pressure system is shown in the picture. The HPP system consist of following components –

- (i) Pressure vessel of desired capacity.
- (ii) Closure(s) for sealing the vessel.
- (iii) Yoke – a device for holding the closure(s) in place while the vessel is under pressure.
- (iv) High pressure intensifier pumps to generate the high pressure

- (v) Pressure and temperature controlling and monitoring system.
- (vi) Product and material handling system.



**Packaging requirements for HPP**

- In HPP, the product is generally treated in its final primary package form resulting a 'secure unit' until the consumer opens it.
- Foods decrease in volume as a function of pressure during compression; almost an equal expansion occurs upon decompression.
- Airtight flexible packages, that can withstand a change in volume corresponding to the compressibility of the product, are needed.
- The packaging must be able to accommodate up to a 15 % reduction in volume and return to its original volume without loss of barrier properties.

### **Packaging requirements for HPP**

In the high pressure processing, the food product is generally treated in its final primary package form resulting a 'secure unit' until the consumer opens it for the consumption. Therefore, the packaging material which is undergoing the high pressure treatment should withstand the pressure inside the vessel. Generally, foods decrease in volume as a function of pressure during compression and almost an equal expansion occurs upon decompression. Airtight flexible packages that can withstand a change in the volume corresponding to the compressibility of the product are needed in HPP system. In general, the packaging material must be able to accommodate up to 15% reduction in volume and return to its original volume without the loss of barrier properties after decompression.



- Packaging materials that are impermeable to oxygen and opaque to light should be used for retaining fresh colour and flavour of HP treated foods.
- Plastic films [Ethylene-vinyl alcohol (EVOH) and polyvinyl alcohol (PVOH) copolymer films] are generally used.
- The use of semi-rigid trays is also possible.
- Vacuum-packed products are ideally suited for HP treatment.
- Metal cans and glass containers are generally not suitable for HPP.

The packaging material those are impermeable to oxygen and opaque to light should be used to retain the fresh colour and flavour of the high pressure treated foods. Because the oxidation reactions are normally in the high pressure processed foods which changes the product characteristics in presence of the oxygen and light, e.g. plastic films - ethylene vinyl alcohol (EVOH) or polyvinyl alcohol (PVOH) copolymer films are generally used. Depending upon the material characteristics, sometimes the semi-rigid containers can also be used. Vacuum packaged products are ideally suited for high pressure treatment. The use of metal cans and glass containers should be avoided because of their rigidity as they can break during the compression.

### HPP products & process parameters

HPP products are commercially available in international markets

Product	Processing Condition
Jam	400 MPa, 20°C
Fruit dressings	10-30 min
Fruit sauces	
Fruit jellies	
Yogurt	
Guajava juice	120-400 MPa, 20°C
	2-20 min
Mandarin juice	300-400 MPa, 20°C
	2-3 min
Sugar impregnated tropical fruits for shelves and ice creams	50-200 MPa
Beer carbonation	100-200 MPa, 20°C
	30 min-1h
Rice cake	400 MPa, 45°C-70°C
	10 min

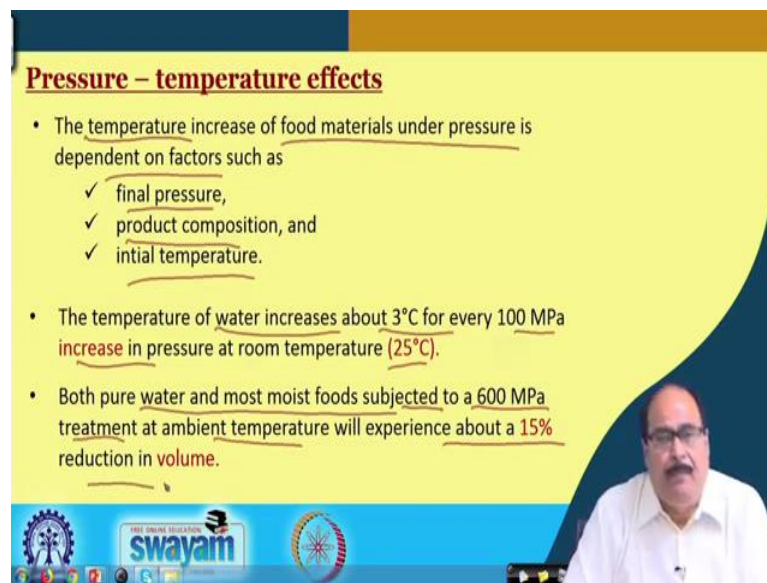
✓ Jams, jellies, juices, salad dressings  
 ✓ Fish, meat products, sliced ham  
 ✓ Rice cakes, and  
 ✓ Yogurt

Other Product (14%)  
 Vegetables Product (32%)  
 Meat product (27%)  
 Sea food product (18%)  
 Juice & Beverage (11%)

## HPP products and process parameters

HPP products are commercially available in international markets. Those products generally are jams, jellies, juices, salad dressings, fish, meat products, sliced ham, rice cake, yogurt, etc. The share of HPP products includes 32% vegetable products, 27% meat products, 16% sea foods, 11% juices and beverages and 14% other products.

Various studies have been reported in literature on HPP treatment of food products using different process combinations i.e. pressure, temperature and time depending upon the requirement of the process. As presented in the Fig., generally 100-300 MPa pressure and comparatively lower holding times are used for the extraction processes. Commercial non thermal pasteurization can be achieved in the range of 400-600 MPa with little higher holding times. If intention is to sterilize the product depending upon its acidity & other factors, high pressures like 700-900 MPa or more with higher holding times are required. This gives an idea about the pressure requirement depending upon the characteristics of the food materials and the desired application.



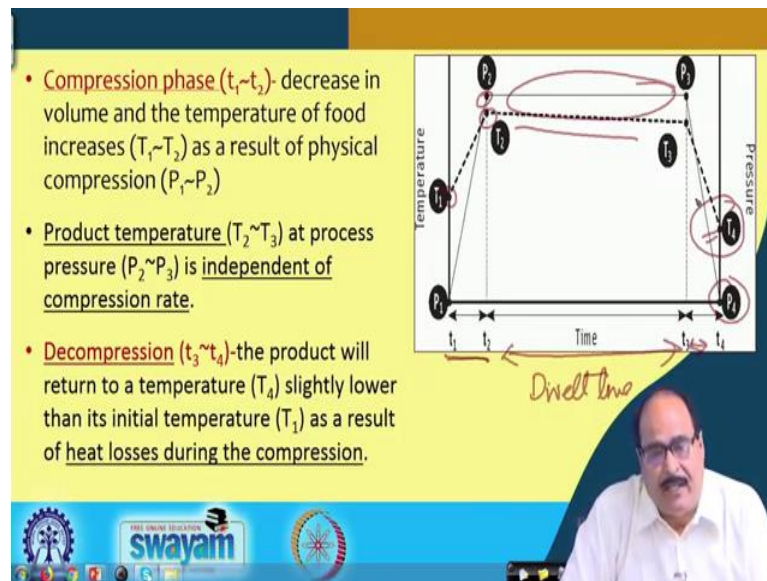
**Pressure – temperature effects**

- The temperature increase of food materials under pressure is dependent on factors such as
  - ✓ final pressure,
  - ✓ product composition, and
  - ✓ initial temperature.
- The temperature of water increases about 3°C for every 100 MPa increase in pressure at room temperature (25°C).
- Both pure water and most moist foods subjected to a 600 MPa treatment at ambient temperature will experience about a 15% reduction in volume.

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## Pressure-temperature effects

The temperature increase of food materials under pressure is dependent on factors such as final pressure, initial temperature and product composition, etc. The temperature of water increases about 3°C for every 100 MPa increase in the pressure at room temperature. Both pure water and most of moist foods subjected to a 600 MPa treatment at ambient temperature will experience about 15 % reduction in volume.



The interrelation of the pressure - temperature against time is shown in the figure. The HPP curve consists of the compression phase, holding phase and decompression phase.

**(i) Compression phase ( $T_1-T_2$ )**

During this phase, the increase in pressure ( $P_1-P_2$ ) increases the temperature of material ( $T_1-T_2$ ) and decreases the volume. The compression rate and the compression time affect the change in the temperature and volume.

**(ii) Holding phase ( $T_2-T_3$ )**

The time during holding phase ( $T_2 - T_3$ ) is called as hold time or commonly known as dwell time. During this phase, the product is held at constant pressure and temperature. However, there is slight decrease in temperature depending upon the system and product constituents. Product temperature ( $T_2-T_3$ ) at process pressure ( $P_2-P_3$ ) is independent of compression rate.

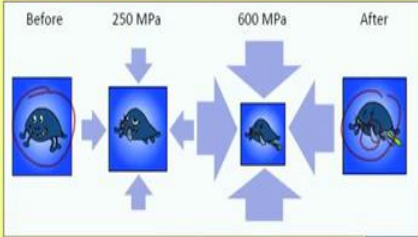
**(iii) Decompression phase ( $T_3-T_4$ )**

The phase between  $T_3$  to  $T_4$  is called as decompression phase, where the pressure is released to its original value of  $P_4$ . Simultaneously, the temperature also drops from  $T_3$  to  $T_4$ , slightly lower than its initial temperature  $T_1$ , because of the heat loss during the compression period.



### Mechanism of microbial inactivation during HPP

- Mechanism
  - ✓ Involve shear force generated membrane disruption
  - ✓ Interruption of cellular function
  - ✓ Localized thermal damage
  - ✓ Protein deformation
- Resistance
  - Spores > Gram +ive > Gram -ive
- pH and  $a_w$  of food affect the lethal effect of pressure



The diagram illustrates the process of microbial inactivation during High Pressure Processing (HPP). It shows a sequence of four stages: 'Before', '250 MPa', '600 MPa', and 'After'. In the 'Before' stage, a microbial cell is shown in its normal, rounded shape. At '250 MPa', the cell is compressed and flattened. At '600 MPa', the cell is further compressed and appears distorted. In the 'After' stage, the cell has returned to its original rounded shape, but it is shown with a cracked membrane and internal damage, indicating that the cell is physiologically inactive despite its restored appearance.

### Mechanism of microbial inactivation during HPP

When the foods are processed under high pressure to extend the shelf life, the microorganisms and enzymes get inactivated or killed. The inactivation of microorganisms during high pressure involves disruption of membrane due to shear force generated. This result into interruption of cellular functions, localized thermal damage and protein deformation.

When the pressure is applied from all the sides, the microbial cell compresses and after decompression, it may again come to its earlier size, but here its activity is ceased because the cell material oozes out, that makes it physiologically inactive.

The resistance of vegetative cells of microorganisms to the high pressure treatment is generally less than the spores. The spores of Gram positive cells are more resistant to that of the Gram negative cells. The pH and water activity of the food affect the lethal effect of the pressure during high pressure processing.

### Effect of HPP on bacteria

- ✓ Treating food samples using HP can destroy both pathogenic and spoilage microorganisms.
- ✓ There is, however, a large variation in the pressure resistance of different bacterial strains.
- ✓ Nature of the medium can affect the response of microorganisms to pressure.
- ✓ The cell walls of Gram negative bacteria are significantly weaker and, therefore, Gram negative bacteria are more pressure-sensitive than Gram positive bacteria.



### Effect of HPP on bacteria

The high pressure destroys both the pathogenic and spoilage microorganisms. However, there is large variation in the pressure resistance of the different bacterial strains. Nature of the medium in which the pressure is applied also affects the response of microorganisms to pressures. The cell wall of Gram negative bacteria are significantly weaker than that of the Gram positive bacteria and therefore, Gram negative bacteria are more pressure sensitive than Gram positive bacteria.

### Effect of HPP on bacterial spores

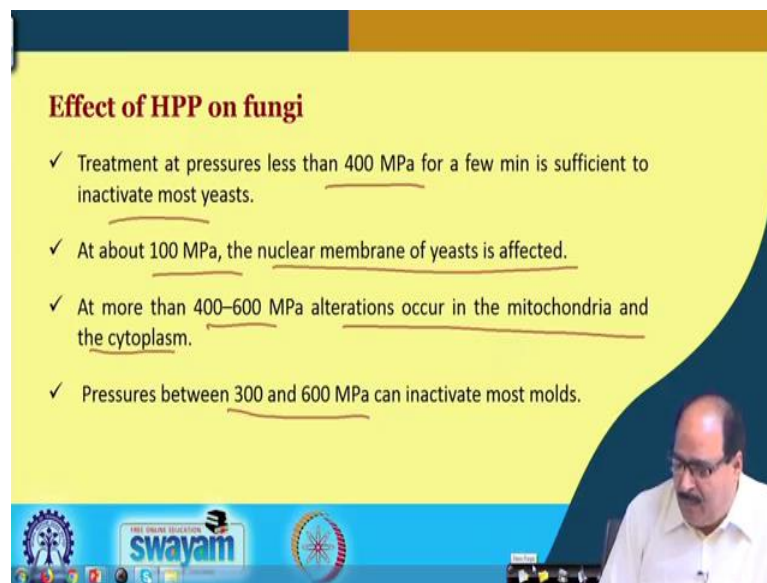
- ✓ Spores are the most pressure-resistant.
- ✓ Very high pressures (>800 MPa) are needed to kill bacterial spores at ambient temperatures.
- ✓ Spores of certain bacterial species might need as high as 1400 MPa for killing/inactivation in low acid foods at ambient temperature.
- ✓ Pressure induced inactivation of bacterial spores is markedly enhanced at temperatures of 50–70°C and perhaps also at/or below 0°C.
- ✓ Bacterial spores can be stimulated to germinate by treatment at relatively low pressures, e.g. 50–300 MPa; germinated spores can then be killed by relatively mild heat treatments or higher pressure treatments.



## Effect of HPP on bacterial spores

Bacterial spores are more resistant to high pressure. Very high pressure (may be more than 800 MPa) are needed to kill bacterial spores at ambient temperature. Even spores of certain bacterial species might need as high as 1400 or 1500 MPa for killing or inactivation particularly in low acid food at ambient temperature. Pressure induced inactivation of bacterial spores is significantly enhanced at temperature of 50– 70°C and perhaps also at/ below 0 °C.

Bacterial spores can be stimulated to germinate by treatment at relatively lower pressure, e.g. 50-300 MPa. Then the germinated spores can be inactivated by relatively mild heat treatments or even higher pressure treatments. It is always advisable to have a combination of pressure and temperature to inactivate spores particularly in low acid food.



**Effect of HPP on fungi**

- ✓ Treatment at pressures less than 400 MPa for a few min is sufficient to inactivate most yeasts.
- ✓ At about 100 MPa, the nuclear membrane of yeasts is affected.
- ✓ At more than 400–600 MPa alterations occur in the mitochondria and the cytoplasm.
- ✓ Pressures between 300 and 600 MPa can inactivate most molds.

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## Effect of HPP on fungi

Fungi are normally lesser resistant than the bacteria. Treatment at pressure less than 400 MPa for a few minute is sufficient to inactivate most of the yeasts. At about 100 MPa, the nuclear membrane of yeast is affected, whereas at more than 400- 600 MPa, alterations in the mitochondria and cytoplasm of the yeast cell are caused. It ultimately causes the destruction of its normal activity. Pressures between 300 and 600 MPa can inactivate most molds.

## Factors affecting sensitivity of microorganisms in HPP

- ✓ Factors that can affect the response of microorganisms, including pathogens, to pressure must be considered so that treatments can be optimized and microbiological safety can be assured.
- ✓ Major factors affecting sensitivity of microorganisms to HP are
  - ✓ pH,
  - ✓ Water activity ( $a_w$ ),
  - ✓ Temperature,
  - ✓ Pressure, and
  - ✓ Holding or Dwell time.



## Factors affecting sensitivity of microorganisms in HPP

Various factors influence the response of microorganism including pathogens to high pressure treatments and obviously, these factors must be considered while optimizing high pressure process parameters or the microbiological safety.

The major factors affecting the sensitivity of microorganisms to HP includes pH, water activity, temperature, pressure and holding or dwell time.

**Table 1.3** Predicted treatment pressure required at various temperatures for a 5-log<sub>10</sub> inactivation of *Escherichia coli* and *Staphylococcus aureus* in poultry meat and milk, for a treatment time of 15 minutes (Patterson and Kipatrick, 1998)

Temperature (°C) during pressure treatment	Estimated pressure (MPa)			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Poultry meat	UHT milk	Poultry meat	UHT milk
10	850	1014	647	749
20	779	966	694	625
30	681	840	735	602
40	544	638	701	583
50	371	392	524	478
60	125	133	177	196

**Table 1.2** Pressures required to achieve a 5-log cycle inactivation ratio for certain microorganisms, for a 15-minute treatment (Patterson et al., 1995)

Microorganism	Pressure (MPa)
<i>Yersinia enterocolitica</i>	275
<i>Salmonella typhimurium</i>	350
<i>Listeria monocytogenes</i>	375
<i>Salmonella enteritidis</i>	450
<i>Escherichia coli</i> O157:H7	680
<i>Staphylococcus aureus</i>	700

Some results from the literature are presented in the Table. The high pressure treatment required for 15 min to achieve 5-log cycle inactivation of *Escherichia coli* and *Staphylococcus aureus* in poultry meat and milk. Increasing temperature led to decrease

in the pressure requirement for 5-log cycle reduction of *E-coli* in both the case of poultry meat and milk. The pressure requirement for the similar inactivation in milk is more than that of the poultry meat. Similar trend was followed for the *Staphylococcus aureus*.

Another study reported the 5-log cycle reduction of certain microorganisms during a 15 minute treatment. It was found that among the different groups of problematic microorganisms in the food, *Staphylococcus aureus* was more pressure resistant than that of the other microorganisms.

**Enzyme inactivation during HPP**

- ✓ Covalent bonds (primary structure) are generally not affected.
- ✓ Tertiary structure are modified followed by a large hydration change.
- ✓ Disruption of hydrophobic and electrostatic interactions leads to changes in quaternary structure.
- ✓ Dense hydrophobic hydration layer is formed leading to volume changes.

$$\frac{d\Delta G}{dP} = -\Delta V = RT \frac{d \ln K}{dT}$$

Normal protein → Denaturation (loss of biological activity) → Denatured protein  
 Reverse activity / Renaturation

### Enzyme inactivation during HPP


Enzymes are also resulted into the deteriorative reactions and their inactivation becomes important criteria for the high pressure stabilization of the food. In addition to microorganisms, process should also be able to destroy certain enzymes. As shown in figure, the  $\Delta G$  is a function of pressure change.  $\Delta G$  indicates weather the protein remains in its native state or denatured as a function of pressure and temperature.

It has been seen that the primary structure of protein generally is not destroyed or not affected by high pressure treatments. Tertiary structures are modified followed by a large hydration change. Disruption of hydrophobic and electrostatic interactions during high pressure processing may lead to certain changes in the quaternary structure and there is a dense hydrophobic hydration layer is formed which will lead to the volume changes. So, by changing the confirmation of the structure, the activity of the enzymes might be reduced.



### Enzyme inactivation by HPP

Enzyme	Observations
Pectin methyl esterase (PME)	<ul style="list-style-type: none"> <li>At 600 MPa, partially (up to 90%) and irreversibly inactivated (which does not reactivate during storage and transportation) in orange.</li> <li>Tomato PME are more pressure resistant; inactivation seems to follow first-order kinetics</li> </ul>
Peroxidase (POD)	<ul style="list-style-type: none"> <li>A treatment of 900 MPa for 10 min at room temperature was needed to cause an 88% reduction of POD activity in green bean.</li> <li>A combination with temperature treatments enhanced the inactivating effect at 600 MPa, but no significant differences were detected at 700 MPa.</li> </ul>
$\alpha$ -amylase	<ul style="list-style-type: none"> <li>Pressure and temperature (upto 400 MPa, 60 °C) showed both synergistic and antagonistic effect on the inactivation.</li> </ul>
Lipoxygenase (LOX)	<ul style="list-style-type: none"> <li>Pressure inactivation of soybean LOX could be accurately described by a first-order kinetic model.</li> </ul>
Polyphenol oxidase (PPO)	<ul style="list-style-type: none"> <li>Is most pressure stable at room temperature.</li> <li>Are very pressure stable, treatments at 800 - 900 MPa are required for activity reduction.</li> </ul>



The stability of the various food enzymes like pectin methyl esterase, peroxidase, alpha amylase, lipoxygenase, and polyphenol oxidase were studied by various researchers (see Table). It is seen that the pH, water activity, and temperature of system influences the stability at various high pressure and temperature ranges. It is found in general that, the polyphenol oxidase and peroxidases are comparatively pressure stable. A pressure treatment at 800-900 MPa is required for their inactivation. Other enzymes like pectin methyl esterase are also equally pressure stable. At 600 MPa pressure, the pectin methyl esterase enzyme partially (up to 90%) and irreversibly inactivated (which does not reactivate during storage and transportation) e.g. tomato PME are more pressure resistant and follow first order kinetics.