



High Hydrostatic Pressure (HPP) in Food Processing: Design Aspects and Applications

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Abstract

There are many well-recognized processing technologies developed worldwide in the field of food science and technology and being commercialized now-a-days for conversion of raw materials into various edible food products. These technologies have gained the worldwide acknowledgement for their potential and versatile applications. More ever, the growing consumers demand for fresh, safe, chemical, additive or preservative free and minimally processed foods has insisted the food scientists and technologists to develop many non-thermal food processing technologies which cause minimum nutritional and sensory qualities impairment in the processed foods. In this endeavour, consumers prefer minimally or non-thermally processed foods rather than the thermally processed one. Today, non-thermal technologies like high pulse electric field (HPEF) processing, high hydrostatic pressure (HPP) processing, food irradiation, ultraviolet light, ultra sound, arc discharge, oscillating magnetic fields, light pulses, plasma, chemicals (ozone, carbon dioxide, argon), and combined methods of these technologies are the major areas of research in the process and food engineering in order to find their suitability for processing of various food products. In the present article, an attempt has been made to give an insight about the high pressure processing of foods, its principle, process design and applications to various food products in order to explore the application of high pressure processing in food industries.

Keywords: Non-Thermal Processing, Cold Isostatic Pressing, Food Standards, High Pressure Processing, High Pulse Processing, Food Safety.

Introduction

Consumers demand for high quality and convenient foods with natural flavour and taste, and greatly appreciate the minimally processed foods. In order to harmonize their demands without compromising the quality and safety of foods, it is necessary to implement or develop novel food processing technologies in food industry. Although high pressure processing was first introduced by Japanese scientists in 1899 and has been used widely with success in chemical, ceramic, carbon allotropy, steel, composite materials and plastic industries for decades, it was only in late 1980's that its commercial benefits became available to the food industries. High pressure processing is similar in concept to cold isostatic pressing of

metals and ceramics, except that it requires much higher pressure, faster cycling, high capacity, and sanitation. Recent progress in high hydrostatic pressure equipment design, combined with increasing consumers demand for high quality, minimally processed, additive-free and microbiologically safe food have ensured the world-wide acknowledgement of the potential for high hydrostatic pressure processing technology in food industry.

Principle of High Hydrostatic Pressure Processing

The basic principle behind high hydrostatic pressure processing is that pressure causes a

decrease in the available molecular space or increase in chain interactions. The reactions involved with formation of hydrogen bonds, are favoured by high pressure, because bonding results in a decrease in volume. Pascal's law states that pressure exerted at any point upon a confined liquid is transmitted in all directions. In high pressure processing, foods are subjected to high pressure by compressing the medium surrounding the foods.

In high hydrostatic pressure processing, the pressurization of materials is carried out in following ways.

- (i) *Cold isostatic pressing (CIP)*: This type of equipment is essentially used to shape powdered materials which is subsequently treated with high isostatic pressure at room temperature. The pressure levels vary in the range of 50 to 600 MPa for industrial applications and the volume of the compacted products varies from several cubic centimetres to more than one cubic metre. The typical cycle time varies from 0.3 to 5 min.
- (ii) *Warm isostatic pressing (WIP)*: It is similar to CIP, whereby isostatic pressure is applied in combination with temperatures ranging from ambient to 200 °C. The WIP is normally used for products that require a chemical reaction during the compression phase. The typical cycle time varies from 10 to 30 min.
- (iii) *Hot isostatic pressing (HIP)*: It is a forming process that uses gas such as argon, nitrogen, helium, or even air at air temperature up-to 2200 °C as pressure medium. This process is mostly used to shape metallic and ceramic powders, for densification of casting, and for improvement of mechanical properties. The typical cycle time varies from 6 to 12 hr.
- (iv) *Quartz growing*: This equipment operates at a temperature of about 400 °C and a pressure of 200 MPa. The typical cycle time varies from 24 to 36 days.
- (v) *Chemical reaction*: This is used for high temperature up-to 350 °C and pressure up-to 200 MPa to increase the desired reaction of the product, such as low-density polyethylene.

A typical high hydrostatic pressure system is shown in Fig. 1. The system consists of a high pressure vessel and its closure, a pressure generation system, a temperature controller and a material handling system.

- (i) *High pressure vessel*: The heart of a high pressure processing system is in many cases, simply a forged monolithic, cylindrical vessel constructed by a low-alloy steel of high tensile strength. The wall thickness is determined by the maximum working pressure, the vessel diameter, and the number of cycles for which the vessel is designed. The required wall thickness can be reduced by using multiplayer, wire-wound, or other pre-stressed designs.
- (ii) *Closures*: Most fast cycling CIP systems use interrupted threaded closures allowing very fast opening and closing of the vessel and hence, minimum vessel down time for loading and unloading. These threaded closures are self-centering and can be automatically opened and closed by means of a hoist device, guiding the closure without any thread friction.
- (iii) *Pressure transmitting medium*: In most current cold applications, the pressure medium is simply water mixed with a small percentage of soluble oil for lubrication and anticorrosion purpose.
- (iv) *Pressure generation*: After all air is removed from the high pressure vessel, the high pressure is generated by direct compression (piston type) (Fig. 1a) or indirect compression type (pump type) (Fig. 1b).
 - (a) In direct piston type compression, the pressure medium in the high pressure vessel is directly pressurized by a piston. This method allows very fast compression but is, in practice, restricted to small-diameter, laboratory or pilot, high-pressure system.
 - (b) The indirect compression method uses a high pressure intensifier to pump the pressure vessel until the desired pressure is achieved. For this method, only static high pressure seals are needed within the high pressure vessel.

(v) *Temperature:* Temperature of the load and pressure medium inside the vessel can be controlled by heating/cooling the entire pressure vessel or by internal heating/cooling in which case the heated/cold source is placed inside the vessel. The simplest execution of external heating uses electric heater bands wound around the vessel.

Types of High Pressure Processing of Foods

Batch type

Batch processing has been trusted as the preferred method for high pressure food treatments, mainly for its cleanliness, flexibility, and technical reasons. This process is necessary for packaged foods. The food is prepared and aseptically filled/sealed in plastic containers, then placed in a pressure

chamber for pressurizing, using pure water as the transmitting fluid. The cycle time depends on the food type and the processing temperature. The chamber is then decompressed, and the cycle begins again.

Batch processing eliminates any risks that the food may be contaminated by lubricants or wear particles from machinery. The equipment doesn't need cleaning between product changes, thus eliminating any danger of cross contamination by food particles. More ever, handling, drying, storage and storage of packages lengthens the overall processing cycle and hence increases the overall cost.

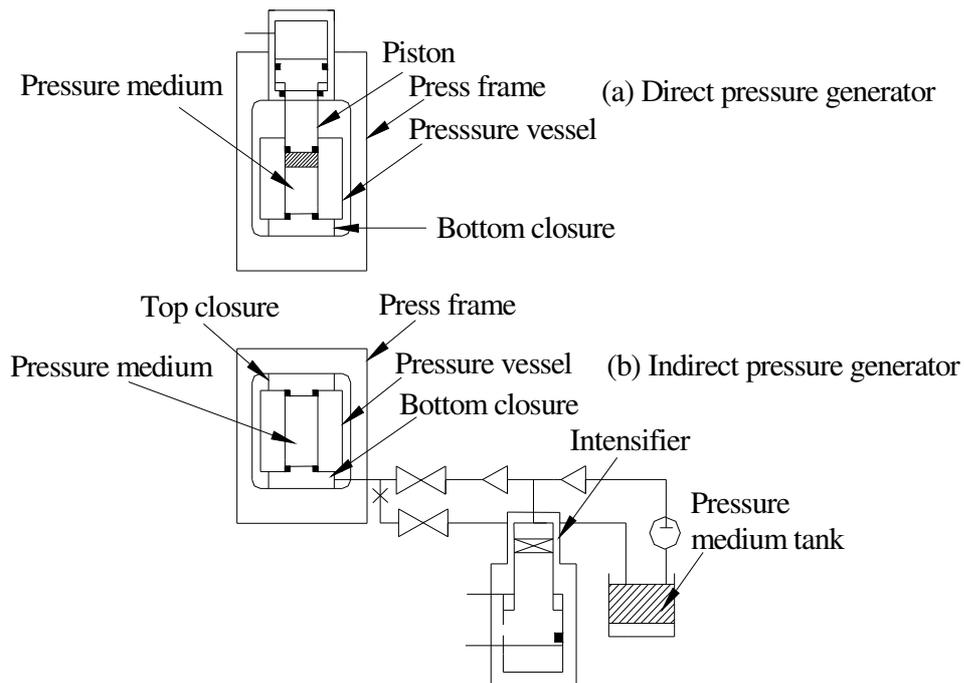


Fig. 1 Methods for the generation of high isostatic pressure

Semi-continuous type

Direct introduction of food into the high pressure chamber is a promising alternative process compared to that of batch one. So far, this is achieved industrial only in a semi-continuous mode, which means that the food is introduced periodically into the high pressure processing chamber. The overall processing cycle includes a

number of discrete steps like filling, pressurizing, holding, decompression and expulsion. The combination of multiple vessels, which sequentially, and which are fed by a central high pressure compressor, can be used to make continuity in the process. Fig. 2 shows a multi-vessel arrangement for food processing.

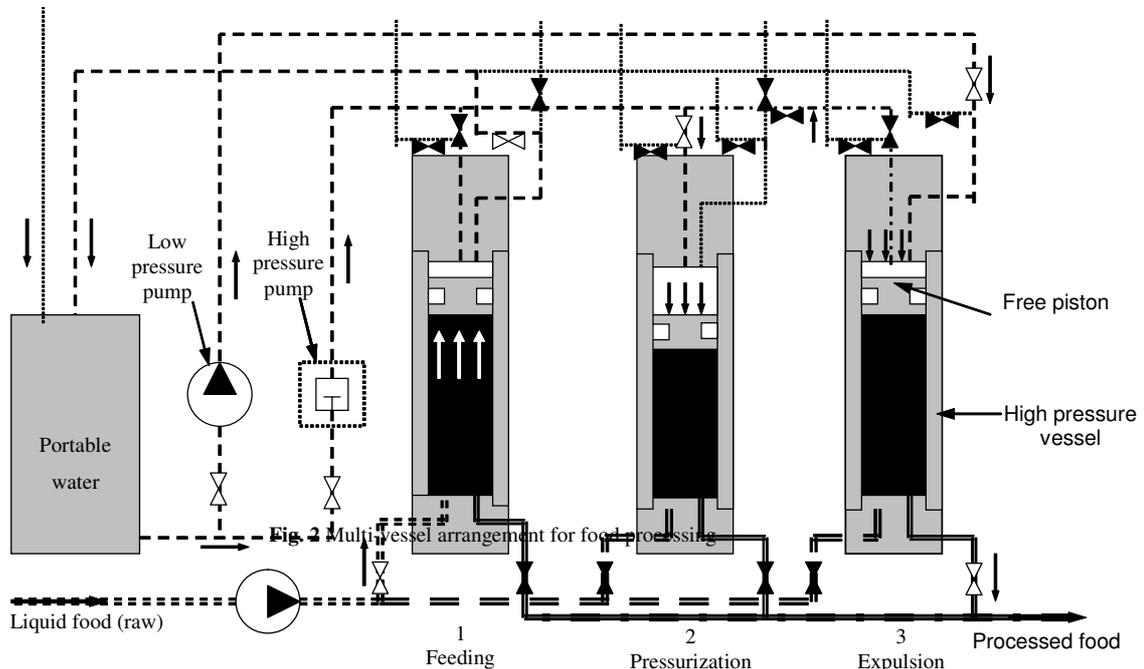


Fig. 2 Multi-vessel arrangement for food processing

Working of High Hydrostatic Pressure Processing

Pressure treatment

Typical size of high pressure vessels available for food processing varies from 100 to 500 litres. The pressure can be controlled and measured throughout the cycle, and accurate repeatability of the cycle parameters can be achieved. The measurement of both pressure and temperature are made directly in the high pressure media to ensure correct readings and control.

Pressurization

Once the product is loaded into the high pressure chamber of the vessel and the vessel is closed, the pressure is increased by using a piston until the desired pressure level is achieved. The pumping unit of fluid into the press consists of standard components working at relatively low pressure, assuring high reliability and minimum cycle time. After pressurization and holding sequence, the vessel is decompressed and the pressurized product is unloaded. Two main methods of pressurization may be used in practice: the direct and indirect method.

Fig. 1a shows a direct pressurization system. In this case, the intensifier is contained within the vessel itself. This intensifier works generally at a lower pressure than the one used in the indirect method. The operating pressure will be the feeding pressure of the intensifier multiplied by the ratio of the piston diameters of the low pressure to the high pressure ones. The pressure vessel and the intensifier represent one unit and this increases the total volume of the vessel to be installed. The high pressure ram has the same diameter as the internal diameter of the pressure vessel. For production units, close control of the vessel diameter is important in ensuring the dynamic sealing of the large corresponding ram. The working volume of the pressure vessel will vary as a function of the pressure to be reached within that vessel, as the ram displaces itself by an amount related to the pressure to be achieved.

Fig. 1b shows the principle of the indirect approach in which pressurization fluid is pumped from the pressure medium tank to the pressure vessel by means of an intensifier. The purpose of such an intensifier is to increase the pressure in the pressure vessel by compressing the fluid and

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the product to be pressurized. In this specific case, the intensifier operates independently from the vessel: i.e. the pressure generator entity in its own right. It also uses small diameter pressurization piston/cylinders, which limit the wear of their dynamic seals. These seals can be quickly and easily replaced where necessary. Connection of the intensifier with the vessel is carried using high pressure tubing.

Pressure level

For pressures lower than 400 MPa, standard high pressure pumps are used for pressurizing. The pressures above 400 MPa will wear standard pump out quickly, making the system more costly. For food processing applications, low pressure pump feeding is incorporated in the system.

Design Aspects of High Hydrostatic Pressure Processing

General considerations

Pressure level

For production purpose, the capacity of the vessel should be large enough in order to make the high pressure processing system cost effective. Operating pressure has a significant effect on processing cost because it increases the investment cost significantly and may reduce holding time required but pressurization and decompression cycles will be increased. In addition, safety and fatigues on stresses and calculated cycle has to be considered. The production vessels are damaged for life times, which are generally between 50000 and one million cycles. In addition, the operating pressure of the vessel may be higher than those applied in most cases in the material industry.

Corrosion

The pressure vessels are commonly pressurized with emulsified water or oil. These fluids have no corrosive action on the vessel, which is typically fabricated from low alloy steel. In order to avoid product contamination, the vessel is pressurized either directly, if possible, or otherwise by using pure water. The internal product contact surfaces of the vessel must, therefore, be protected against corrosion by the food grade material itself, the

water or any other materials such as cleaning solutions that may be used.

Apparatus flexibility

High pressure system can be used to handle different food products irrespective of their state or shape. The system must be designed in such a way that it requires the minimum installation space. Connections to utilities must be quick and simple. The energy consumption related to the pressurizing group and in some cases, temperature generators must be as low as possible. Sample loading and unloading must be easy.

Operating parameters

Many microorganisms and enzymes are inactivated in the pressure range of 400-600 MPa. However, in some cases, destruction of particular spores requires even higher pressures. It is also cheaper to process at high pressure since the processing time and the number of processing cycle can be reduced. The processing cost is a function of time and pressure indicates that processing of foods at 400 MPa with a 10 min hold time is twice as expensive as processing at 800 MPa with no holding time. The combination of pressure, pressure holding time and temperature at which the product is processed must, therefore, be evaluated carefully. The ideal mix for each specific product is the most cost effective processing system. In addition, the operating temperatures above ambient may require lower pressure and/or a short cycle time. Some tests may also require for testing the foods under combined treatment of pressure and temperature. In this case, particular attention must be given to selection of the high pressure vessel material.

Operating parameters control

There is not any difficulty in either controlling or measuring the system pressure for high pressure system, even when the pressure exceeds 100 MPa. Rapid pressurization can be achieved within about one minute and decompression can be even faster. The cycles may be manual or fully automatic depending on the system design. With regard to the system working under combined pressure and temperature treatment, attention

must be paid to the interaction between these two parameters. A unique design consists of internally heating or cooling the pressure chamber, thereby directly controlling the temperature of the samples. This allows simultaneous control of both pressure and temperature during the complete cycle. Furthermore, recording of the actual operating parameters can be used to confirm accurate cycle reproducibility.

Design of high pressure vessels

In-container process

Numerous high-pressure vessels are available for chemical and food applications. Depending on versatility in applications of high pressure processing, the vessels have various sizes and operating pressures. Table 1 shows examples of some of the existing high pressure vessel.

In-container pressure processing system involves the pressurization of a number of packages already filled with food material to be treated. Capacity of the system depends upon some of the characteristics, which are summarized below.

- (i) *Operating pressure:* The higher the operating pressure, the greater the capacity of the high pressure pump should be in order to achieve the operating pressure within the specified time.
- (ii) *Shape and size of the vessel:* The filling of the container must be optimum in the sense that it should contain a minimum amount of air or headspace. The presence of air can drastically increase the time to complete the pressurization and the risk of rupture of the container during pressurization. As the compressibility of food is very close to that of the pressurization fluid, it is important, for production capacity reasons, that maximum number of packing units should be pressurized in each compression cycle. The size of the vessel must take into account the required production capacity and also the available flow of the pumping system.
- (iii) *Vessel loading and unloading:* The system for loading and unloading should be automated. It has to be designed in such a way that these

operations occur quickly in order to avoid any affect on the fast cycle time. For instance, it is common practice to install sterilizers horizontally and pressure vessels, in most of the cases, vertically.

- (iv) *Shape and size of food packages:* In a high pressure system, the pressure generator is designed to work up-to at least maximum operating pressure of the vessel. As most food materials have compressibility very close to that of the pressurized fluid, the pressurization time for a given vessel will be independent of the amount of food material to be processed. It is therefore important that the size and shape of the food packaging both the studied to be optimized the pump- time.

Bulk process

Bulk process is applicable only to a food that is capable of being pumped. The bulk process offers the advantages that the system is much less significant than in case of the in-container process. However, the bulk process requires an aseptic design of special high pressure components. In fact, there is no established rule to use in choosing between an in-container and a bulk process apart from the basic one of whether the food product is pumpable or not. More ever, if the batch and semi-continuous processes are compared, it can be observed that there is an economic advantage of about 25% for the semi-continuous system. The bulk process requires a specific system to fill the food product into the pressure vessel without having any influence on the quality and the constitutions of the food. Also, the maximum amount of product must fill the vessel and the presence of air must be avoided. Furthermore, the food remains in contact with the vessel surfaces, which should be of non-corrosive and food grade type. The high pressure pumping unit pressurizes water that has to be separated from the food product by a reliable system. The pressurized food is discharged from the vessel through a suitably designed aseptic high pressure valving unit that allows for high flow rates without damaging the product. The bulk process can be converted into a semi-continuous one by utilizing

multiple vessels in parallel depending on the number of vessels required, their capacity, cycle time, and cost.

Applications of High Pressure on Foods

The ability of the high pressure to inactivate micro-organisms and spoilage catalyzing enzymes, whilst

Table 1 Example of existing high pressure vessels

Example	Maximum working pressure (MPa)	Diameter (mm)	Length (mm)	Internal volume (litre)
1.	100	1700	4000	9000
2.	200	1000	4000	3150
3.	410	600	4500	1250
4.	550	600	2500	700
5.	690	250	750	37
6.	1030	100	1000	8.5
7.	1380	90	350	3.5

retaining quality attributes has encouraged Japanese and American food companies to introduce high pressure processed foods in their super markets. The first high pressure processed food was introduced to the Japanese market in 1990 by Meidi-ya, who have been marketing a line of jams, jellies, and sauces processed and packaged without application of heat. Other products include fruit juices, rice cakes, and raw squid in Japan; fruit juices like apple and orange in France and Portugal; and guacamole and oysters in US. In addition, the high-pressure processing can result in new food products acquiring novel structure and texture, and therefore, can be used to develop novel food products or to enhance the functionality of certain food ingredients.

Food Protein

Protein denaturation

Application of high pressure denatures protein depending on protein type, processing conditions, and applied pressure. The high pressure makes peptide bonds of the protein more readily available for hydrolysis. As a result of protein denaturation, the protein solubility decreases and biological properties are lost. Crystallization of protein is no longer possible, and viscosity and optical rotation increase. The increase in viscosity results in more asymmetry of protein. This exposes more

hydrophobic residues resulting in the decreased solubility of protein.

During high pressure induced protein denaturation, proteins may precipitate. The changes are reversible in the pressure range of 100 to 300 MPa and irreversible for pressures higher than 300 MPa. The denaturation may be due to the destruction of hydrophobic and ion pair bonds, and unfolding of molecules. At higher pressure, oligomeric proteins tend to dissociate into subunits, becoming vulnerable to proteolysis. Monomeric proteins do not show any changes in proteolysis with increase in pressure (Thakur and Nelson, 1998).

High pressure effects on proteins are related to the rupture of non-covalent interactions within protein molecules and to subsequent reformation of intra and inter molecular bonds within or between the molecules. The quaternary structure of protein is mainly held by hydrophobic interactions, which are very sensitive to pressure. Significant changes in the tertiary structure are observed beyond 200 MPa. However, a reversible unfolding of small proteins such as ribonuclease A occurs at higher pressures (400 to 800 MPa), showing that the volume and compressibility changes during denaturation are not completely dominated by the hydrophobic effect. Changes in the secondary structure occur above 700 MPa,

leading to irreversible denaturation (Balny and Masson, 1993).

Skim milk protein

Application of high pressure destabilizes casein micelles in reconstituted skim milk. The size distribution of the spherical casein micelles decreases from 200 to 120 nm; maximum changes occur in the pressure range of 150 to 400 MPa at 20 °C. The pressure treatment reduces turbidity and enhances lightness, which leads to the formation of a virtually transparent skim milk (Shibauchi et al., 1992; Derobry et al., 1994). The gels produced from high-pressure treated skim milk show improved rigidity and gel breaking strength (Johnston et al., 1992). The pressure treatment at 25 °C considerably reduces the micelle size, while pressurization at higher temperature progressively increases the micelle dimensions. The size of casein micelles reduces slightly at 100 MPa, with slightly greater effect at higher temperature or longer pressure treatment. At pressure greater than 400 MPa, the casein micelles disintegrate. The effect is more rapid at higher temperature although the final size is similar in all samples regardless of the pressure or temperature. At 200 MPa and 10 °C, the casein micelle size decreases slightly on heating, whereas, at higher temperature, the size increases as a result of aggregation of casein micelles. The size of casein micelles increases by 30% upon high-pressure treatment of milk at 250 MPa and micelle size drops by 50% above 400 MPa (Drake et al., 1997).

The high-pressure treatment of milk at 100 to 600 MPa considerably leads to solubilization of α s1- and β -casein, which may be due to the solubilization of colloidal calcium-phosphate and disruption of hydrophobic interactions. On storage of high pressure treated milk at 5 °C, the dissociation of casein is largely irreversible, but at 20 °C, considerable reassociation of casein occurs. The hydration of the casein micelles increases on pressure treatment of 100 to 600 MPa due to induced interactions between caseins and whey proteins. Pressure treatment increases the level of α s1- and β -casein in soluble phase of milk and produces casein micelles with properties different

than those in untreated milk. The casein micelle size is not influenced by pressure lower than 200 MPa, but the pressure treatment at 250 MPa increases the micelle size by 25%, while pressure treatment of 300 MPa or greater, irreversibly reduces the size to 50% of that in untreated milk (Hinrichs and Rademacher, 2004).

β -lactoglobulin

Pressure treatment of β -lactoglobulin at 450 MPa for 15 min reduces its solubility in water. The high-pressure induces extensive protein unfolding and aggregation when BSA is pressurized at 400 MPa. β -lactoglobulin appears to be more sensitive to pressure than α -lactalbumin. The state of aggregation and thermal gelation properties of pressure-treated β -lactoglobulin occurs immediately after depressurization and after storage for 24 hr at 5 °C. Pressure treatment at 150 MPa for 30 min or pressure higher than 300 MPa for 0 or 30 min forms the soluble aggregates of β -lactoglobulin (Huppertz et al., 2004b). Use of high-pressure (510 MPa for 10 min at 8 or 24 °C) induces unfolding of β -lactoglobulin and characterizes the protein structure and surface-active properties. The secondary structure of the protein processed at 8 °C appears to be unchanged, whereas α -helix structure is lost at 24 °C. High-pressure in the range of 100 to 300 MPa, β -lactoglobulin AB completely hydrolyzes by pronase and α -chymotrypsin. The activation volume for the denaturation of β -lactoglobulin decreases with increase in temperature, and the activation energy increases with pressure up-to 200 MPa, beyond which it declines (Galazka et al., 1997). Isobaric (200-800 MPa) and isothermal (-2 to 70 °C) denaturation of β -lactoglobulin and α -lactalbumin of whey protein follows third and second order kinetics, respectively. Isothermal pressure denaturation of both β -lactoglobulin A and B doesn't differ significantly and increase in temperature increases the denaturation rate (Hinrichs and Rademacher, 2004).

Soya proteins

Gelation of soya proteins induces at minimum pressure treatment of 300 MPa for 10 to 30 min

and the gels formed are softer with lower elastic modulus in comparison with heat-treated gels. The treatment of soya milk at 500 MPa for 30 min changes it from a liquid state to a solid state, whereas at lower pressure, the milk remains in a liquid state, but improves its emulsifying activity and stability (Okamoto et al., 1990). Application of high pressure (200–600 MPa) on soya protein isolated at pH 8.0 increases the protein hydrophobicity and aggregation, and reduces the free sulfhydryl content and a partial unfolding of α -conglycinin (7S) and glycinin (11S) occurs, whereas at pH 3.0, the protein denatures partially and insoluble aggregates are formed (Zhang et al., 2005).

Soybean whey, a by-product of tofu manufacture, is a good source of peptides, proteins, oligosaccharides, and isoflavones. High pressure increases the antioxidative activity of soy whey protein, but decreases the antioxidative activity of the hydrolysates (Prestamo and Penas, 2004). The high pressure treatment enhances the pH of the protein hydrolysates. The pressure treatment (100 and 200 MPa, 15 min, 37°C) hydrolyses the soya whey protein by pepsin, trypsin, and chymotrypsin. The highest level of hydrolysis occurs at a pressure treatment of 100 MPa. After the hydrolysis, the formation of 5-peptides under 14-kDa with trypsin, chymotrypsin, 11-peptides and pepsin are observed (Penas et al., 2004).

Enzymes

Enzymes are a special class of proteins in which biological activity arises from active sites. The changes in the active site or protein denaturation can lead to loss of activity or changes the functionality of the enzymes. In addition to conformational changes, the enzyme activity can be influenced by pressure-induced decompartmentalization. Pressure induced damage of membranes facilitates enzyme substrate contact. The resulting reaction can either be accelerated or retarded by the pressure application.

Pectin methylesterase

Pectin methylesterase (PME) normally tends to lower viscosity of fruit products and adversely affect their texture. Hence, its inactivation is a

prerequisite for preservation of such products. The inactivation of PME in orange juice is dependent on applied pressure, holding time, pH, and total soluble solids (Basak and Ramaswamy, 1996). Pressure in excess of 500 MPa fastens the inactivation rate for economic viability of the process. The combined effect of pressure and temperature on inactivation kinetics of strawberry PME follows a fractional-conversion model (Binh et al., 2002a, 2002b). Purified strawberry PME is more stable towards high-pressure treatments than PME from oranges and bananas. Under ambient pressure, tomato PME inactivation rate increases with temperature, and the highest rate is obtained at 75 °C. The inactivation rate dramatically reduces as soon as the processing pressure is raised beyond 75 °C. The high-pressure inactivation kinetics of PME isolated from a variety of sources shows that PME from a microbial source is more resistant to pressure inactivation than from orange peel (Riahi and Ramaswamy, 2003). Almost a full decimal reduction in activity of commercial PME is achieved at 400 MPa within 20 min. The optimal temperature for tomato PME shifts to higher values at elevated pressure compared to atmospheric pressure, creating the possibilities for rheological improvement. The inactivation kinetics of carrot PME follows first order kinetics over a pressure and temperature range of 650- 800 MPa, and 10-40 °C, respectively (Balogh et al., 2004; Castro et al., 2006).

Pectinesterase

The presence of pectinesterase (PE) reduces the quality of citrus juices by destabilizing the clouds (Goodner et al., 1998). The pressure higher than 600 MPa causes instantaneous inactivation of the heat labile form of the enzyme, but does not inactivate the heat stable form of PE in case of orange and grapefruit juices. Orange juice pressurized at 700 MPa for 1 min has no cloud loss for more than 50 days. The combined pressure-temperature over a pressure and temperature range of 0.1 to 900 MPa and 15 to 65 °C, respectively inactivates the labile fraction of orange PE. Pressure and temperature

dependence of the inactivation rate constants of the labile fraction is quantified using Eyring and Arrhenius relations. The stable fraction is inactivated at a temperature higher than 75 °C. Acidification (pH 3.7) enhanced the thermal inactivation of the stable fraction, whereas the addition of Ca²⁺ ions (1M) suppressed the inactivation. At pressure up to 900 MPa, an antagonistic effect of pressure and temperature on the inactivation of the stable fraction is observed. The combined heat and pressure treatment on the inactivation of purified carrot PE follows a fractional-conversion model. The thermally stable fraction of the enzyme could not be inactivated. At lower pressure of 300 MPa and higher temperature of 50 °C, an antagonistic effect of pressure and heat is observed (Ly-Nguyen et al., 2003).

Polygalacturonase

High pressure induced conformational changes in polygalacturonase (PG) reduces the substrate binding affinity and enzyme inactivation (Eun et al., 1999). In combination of pressure-temperature treatment (5–55 °C/100–600 MPa), the inactivation of the heat labile portion of purified tomato PG follows first order kinetics. The heat stable fraction of the enzyme shows pressure stability very similar to that of heat labile portion. The effect of high-pressure is identical on heat stable and heat labile fractions of tomato PG (Fachin et al., 2004). The isoenzyme of PG is detected in thermally treated (140 °C for 5 min) tomato pieces and tomato juice, whereas, no PG is marked in pressure treated tomato juice or pieces. At atmospheric pressure, the optimum temperature for enzyme is 55–60 °C and it decreases with increase in pressure. The enzyme activity decreases with increase in pressure at a constant temperature. The ability of high pressure treatment in inactivation of lipoxygenase, PE and PG indicates that the magnitude of the applied pressure has a significant effect on lipoxygenase PE and PG activity, with complete loss of the activity at 800 MPa (Shook et al., 2001).

Polyphenoloxidase and Peroxidase

Polyphenoloxidase (PPO) and peroxidase (POD), which are responsible for colour and flavour loss can be inactivated by a combined treatment of pressure and temperature (Gomes and Ledward, 1996). The pressure treatment (100–800 MPa for 1–20 min) on commercial PPO enzyme available from mushroom, potato, and apple has limited inactivation over grape PPO using pressure in the range of 300 to 600 MPa. In order to reach complete inactivation of grape PPO, a high-pressure treatment with a mild heat treatment (40–50 °C) is necessary. The pressure stabilities of PPO for apple, grape, pear, and plum are observed at pH 6-7. The inactivation of PPO from apple, grape, and pear at room temperature (25 °C) becomes noticeable at approximately 600, 700, 800 and 900 MPa, respectively, and follows the first order kinetics (Castellari et al., 1997). The inactivation of high pressure in the range of 100 to 600 MPa, combined with thermal treatment in the temperature range of 0 to 60 °C on POD and PPO develops shelf stable red grape juice. The lowest POD (55.75%) and PPO (41.86%) activities are obtained at 60 °C, with pressure at 600 and 100 MPa, respectively. Mushroom PPO is highly pressure stable. Exposure to 600 MPa for 10 min reduces PPO activity by 7%; further exposure has no denaturing effect. Compression for 10 and 20 min up-to 800 MPa reduces the activity by 28% and 43%, respectively. Thermal and/or high-pressure inactivation of grape PPO follows first order kinetics. A third degree polynomial describes the temperature/pressure dependence of the inactivation rate constants. Pressure and temperature act synergistically, except in the high temperature (e.g. 45 °C) to low pressure (e.g. 300 MPa) region, where an antagonistic effect is marked (Rapeanu et al., 2005).

Papain

Increasing pressure gradual reduces the activity of papain enzyme. A decrease in activity of 39% is observed when the enzyme solution is initially activated with phosphate buffer (pH 6.8) and subjected to 800 MPa at ambient temperature for

10 min, while 13% of the original activity remained when the enzyme solution is treated at 800 MPa at 60 °C for 10 min (Gomes et al., 1997). In Tris buffer at pH 6.8 after treatment at 800 MPa and 20°C, papain activity loss was approximately 24%. The inactivation of the enzyme is because of induced change at the active site causing loss of activity without major conformational changes.

α-amylase

The effects of pressure and temperature on activity of three different α-amylases from *B. subtilis*, *B. amyloliquefaciens*, and *B. licheniformis* indicates that the changes in conformation of *B. licheniformis*, *B. subtilis*, and *B. amyloliquefaciens* amylases occur at pressure levels of 110, 75, and 65 MPa, respectively. *B. licheniformis* amylase is more stable than amylases from *B. subtilis* and *B. amyloliquefaciens* to the combined heat/pressure treatment (Weemaes et al., 1996). Pressure inactivation of amylase in apple juice is significantly influenced by pH, pressure, holding time, and temperature.

Dairy Products

Application of high pressure on milk was initiated with a view to develop an alternative process for pasteurisation. It is well established that the pressure inactivates microorganisms such as *L. monocytogenes*, *S. aureus* and *L. innocua* either naturally present in milk or introduced in milk. In addition to the microbial destruction, the effect of high pressure on protein structure and mineral equilibrium suggest the different applications of high pressure processing on dairy products including microbiological stabilisation of milk, processing of milk for cheese and yoghurt, and preparation of milk products.

(In)activation of micro-organisms and enzymes/vitamins

High pressure processing has been widely used to (in)activate many microorganisms and enzymes/vitamins in milk. Pressure in the range of 250 to 450 MPa for 0 to 80 min at 3 or 21 °C inactivates the microorganisms present in raw milk (Pandey et al., 2003). It has been observed that there is a

5-6 log cycle reduction in microbial growth when milk is treated at a pressure of 680 MPa for 10 min at room temperature. Pressure treatment to milk at 400 MPa for 3 min is observed to extend the shelf-life of milk. At this condition there is no significant variation in the content of B1 and B6 vitamins.

Pressures treatment in the range of 200–500 MPa for 60 min at 20 °C are effective for the destruction of pathogen such as *L. monocytogenes*, *E. coli* and *S. enteritidis* and *E. coli* are found to be more sensitive than indigenous micro-flora (Black et al., 2005). High-pressure treatment in the range of 200 to 1000 MPa of pasteurized milk at 63 °C for 30 min reduces the microbial count. Pressure at 300 MPa reduces a 4 log-cycle reduction of microorganisms in milk and subsequently increases the shelf-life up-to 25, 18 and 12 days at 0, 5 and 10 °C, respectively (Mussa and Ramaswamy, 1997).

High pressure treatment of 250 to 500 MPa for 5 min at 20 °C with nisin inactivates gram-positive bacteria. The gram-negative bacteria are more sensitive to high pressure, either alone or in combination with sin, than gram-positive bacteria. Pressure in the range of 300 to 600 MPa has little effect on α-lactoglobulin, while levels of α-lactoglobulin reduces in whey indicating that the denaturation of the α-lactoglobulin is possible above 600 MPa (Pandey and Ramaswamy, 1998; Brooker et al., 1998).

Rennet coagulation of milk

The rennet coagulation time of milk is affected by high pressure treatment with the nature and magnitude of the effect depending on the pressure applied. The pressure in the range of 200–400 MPa decreases the rennet coagulation time (Needs et al., 2000). The coagulation time remains unchanged for pressure treatment less than 150 MPa, whereas it decreases at higher pressure up-to 670 MPa. The coagulation time in the pressure range of 500-800 MPa is higher than the pasteurized milk treated at 72 °C for 15 s. Pressure treatment in the range of 250–600 MPa at 5 or 10 °C reduces the rennet coagulation time. In milk without kio3, the coagulum strength is the highest

after the pressure treatment at 250 or 400 MPa, whereas in milk with KIO_3 , it is highest at the pressure treatment of 400 MPa (Zobrist et al., 2005).

Curd forming and firming

Accelerated rate of curd formation and firming of rennet milk is observed at pressure application of 400 MPa (Ohmiya et al., 1987). There is an increase curd firming rate below 200 MPa. Gel firmness remains unaffected below 200 MPa, but increases at 300 MPa. The rate of curd formation is highest at 200 MPa, but slightly decreases on pressure treatment at 400–600 MPa. Higher curd firmness is observed for the milk treated under high pressure. The decrease in pressure from 200 to 500 MPa at temperature of 3 to 21 °C and holding time of 10 to 110 min decreases the water holding capacity and increases the gel strength of rennet curd. The pressure of 280 MPa at temperature of 9°C for 40 min results in the highest water holding capacity (40%) and highest gel strength (0.47 N).

Cheese ripening, yield, rheology and microstructure

Milk treated with 50 MPa for 3 days accelerates the ripening of cheddar cheese and increases the proteolysis of milk protein. Milk treated with 50 MPa for 3 days at 25 °C accelerates the ripening of commercial cheddar cheese due to degradation of αS1 -Casein and accumulation of αS1 -1-Casein (O'Reilly et al., 2000). Cheddar cheeses treated at pressures higher than 400 MPa releases free amino acids at significantly lower rate than the control. No acceleration in free amino acid development is observed at lower pressure. Pressure treatment doesn't accelerate the rate of texture breakdown. On the contrary, pressure treatment at 800 MPa reduces time-dependent texture change of cheese.

Pressure treatment in the range of 300–600 MPa increases the cheese yield due to denaturation of whey proteins and increases moisture retention. Higher moisture content of cheese made from high-pressure treated milk is due to the fact that casein molecules and fat globules may not aggregate closely and may allow moisture to be trapped in

cheese. The pressure treatment of milk at 400 MPa for 15 min at 20°C increases the cheese yield by 2% (db).

The interaction of high-pressure treatment at 400 MPa for 5min at 21 °C and storage time on non-expressible serum per gram protein is observed in reduced-fat mozzarella cheese. The interaction levels are higher in high-pressure treated cheese than unpressurized sample. High pressure treatment also decreases the *L*, *a* and *b*-values significantly after storage for 1 day, but no effect is observed after 75 days. The composition, pH, proteolysis, rheological properties of the unheated cheese, flowability and stretchability are not significantly affected (Sheehan et al., 2004).

High pressure-treated cheese showed a more continuous/homogeneous protein matrix than non-pressurised cheeses in Confocal scanning light microscopy. The fat phase is also affected, after 15 min of treatment at 450 MPa, where fat globules are observed to lose their circularity and large pools of fat. A more continuous microstructure even after 35 s of pressure treatment post-pressurisation of Cheddar cheese curd is observed.

Fruits and Vegetables

Fruits contain high range of water, ranging from 80 to 90% and a very small amount of protein and fat. The polysaccharides cellulose, hemicellulose and pectic substances are the structural components of fruits. Various sugars are found in fruits, whose content varies in different fruits. Fruits also contain some free organic acids like malic acid, citric acid, and tartaric acid. Fruits are good source of vitamins. Some fruits are very valuable as source of ascorbic acid, citrus fruits being excellent sources of Vitamins, Strawberries, melons and tropical fruits are also good sources of ascorbic acid. Yellow fruits contain carotenoids. Minerals like sulphur, phosphorus, iron and calcium are found in fruits. Some fruits contain a combination of various pigments; orange, for example, in addition to carotenoids contain some chlorophyll.

Orange Juice

High pressure treatment to orange juice at 350 MPa for 1 min at 30 °C results good quality juice with more than 2 months shelf life under refrigeration storage (Takahashi et al., 1998). The storage of orange juice after pressure treatment of 600 MPa for 1 min at 5 °C extends the shelf-life of the orange juice up to 20 weeks at 0 °C without any change in physicochemical and sensory qualities. More ever, there are minor changes after 12 weeks when the samples are stored at 10 °C.

Cloud stabilization in freshly squeezed orange juice occurs at pressure treatment of 700 MPa for 1 min, thereby increasing the shelf-life up-to 90 days under refrigeration conditions. Pressure treatment at 400 MPa for 10 min exhibits acceptable quality of orange juice during storage for 150 days at room temperature (Sanchez et al., 2004). Pressure treatment at 500 or 800 MPa for 5 min, and storage up-to 21 days at 4 °C causes no significant difference in antioxidative capacity, Vitamin C, sugar and carotene content. At pressure level of 500 MPa for 5 min at 35 °C, lower loss of ascorbic acid in orange juice is observed than in conventionally pasteurized juices at 80 °C for 30 s. Pressure range of 350 to 450 MPa at 40 to 60 °C for 1 to 5 min increases the extraction of flavanones. At 600 MPa pressure for 4 min at 40 °C, the rate of degradation of ascorbic acid is lower in orange juice treated with high pressure, which leads to better retention of antioxidative activity when compared with the juice pasteurized in a conventional way by using heat (Bull et al., 2004).

Lemon and Strawberry juice

At the pressure treatment of 450 MPa for 2, 5 or 10 min, no fungus growth is detected in the pressure treated lemon juice sample, whereas the control samples are spoiled due to growth of yeast and filamentous fungi after 10 days (Donsi et al., 1998). In the pressure range of 200 to 500 MPa, no major change in strawberry aroma profile is observed, whereas a pressure of 800 MPa induces significant change in the aroma profile (Lambert et al., 1999).

Guava and Raspberry puree

At 600 MPa pressure at 25 °C for 15 min, guava puree can be stored up-to 40 days at 4 °C without any change in colour, pectin and ascorbic acid content (Gow and Hsin, 1996, 1998,1999). High pressure treatment in the range of 250–400 MPa, pressurization and depressurization treatments causes a significant loss of strawberry PPO (60%) up-to 250 MPa and POD activity (25%) up-to 230 MPa. Optimal inactivation of POD is obtained at 230 MPa and 43 °C in strawberry puree. The highest stability of the anthocyanin content in raspberry puree is observed when the puree is pressurized in the pressure range of 200 to 800 MPa, and stored at 4 °C (Winai et al., 2005).

Fresh cut pineapple

Pressure treatment of 340 MPa for 15 min extends the shelf life of press cut pineapple. The decimal reductions of surviving bacteria are 3.0, 3.1, and 2.5 at 4, 21 and 38 °C, respectively. Pressure treated pineapple pieces has less than 50 cfu/g total plate, yeast and mold counts (Aleman et al., 1994).

Lychee

Pressure treatment of lychee at 200–600 MPa for 10 or 20 min at 20–60 °C causes less loss of visual quality in both fresh and syrup-processed lychee than thermal processing. Pressure treatment at 200 MPa increases the POD activity, whereas further increase in pressure doesn't affect the activity of the POD. The combined effect of pressure and temperature on the PPO activity is more marked at longer treatment time. Pressurization at 600 MPa at 60 °C for 20 min causes extensive inactivation of the POD and PPO in fresh lychee, over 50% and 90%, respectively.

Strawberry jam

Pressure treatment of 400 MPa at room temperature for 5 min produces good quality jam. The texture of the jam is similar to conventionally prepared jam. More ever, the pressure- treated jam has better quality than heat-treated jam. The pressure treated jam could be stored at

refrigeration temperature with minimal loss in sensory and nutritional characteristics up to 3 months (Watanabe et al., 1991).

Carrot, and tomatoes and broccoli

High pressure in the range of 500 to 800 MPa for 25 or 75°C doesn't affect the chlorophyll *a* and *b* content in broccoli, lycopene and α -carotene in tomato, and the antioxidative activities of water soluble carrot and tomato homogenates. The water retention and glucose retardation index of tomato pulp increases and extractability of carotenoids from coarse carrot homogenates reduced (Butz et al., 2002).

Animal Products

Animal product is rich in protein and contains all the essential amino acids. Phosphorous, copper and iron are present in significant amount. Meat contains about 15-20% protein. The lean meat contains 20-22% protein. Of the total nitrogen content of meat, approximately 95% is protein and 5% is smaller peptides and amino acids. The fat content in meat varies from 5 to 40% with the type, breed, feed and age of the animal. The cholesterol content is about 75 mg per 100 g. Glycogen and glucose are found only in small quantities in meat. Meat contains the protein hydrolyzing enzyme, cathepsins which are responsible for increasing tenderness of meat during ageing.

Beef meat (minced)

Pressure treatment above 200 MPa for 20 min at 20 °C reduces 5-log cycles or more for *P. fluorescens*, *C. freundii*, *L. innocua* in beef minced meat. Processing above 400 MPa completely inactivates all the microorganisms such as *Pseudomonas*, *Lactobacillus*, *Coliforms*, except for the total flora, which reduces by 3 to 5 log cycles (Carlez et al., 1993; 1994).

Beef meat and slices

Pressure processing of beef meat at 130–520 MPa for 4.3 min reduces the total flora, and delays the microbial growth by 1 week. The pressure treatment at 130 MPa improves the meat colour by increasing redness, which is maintained for the first 3 days of storage at 4°C, without affecting

the microbiological quality. Pressure treatment at 100 MPa for 10 to 15 min reduces the shear strength and pink colour in the sliced beef (Jung et al., 2003).

Beef, beef muscle and mutton

High-pressure treatment in the range of 100 to 300 MPa modulates the proteolytic activities of beef to improve its quality increasing the free amino acid content. Tryptic digestibility of the extract of beef is increased at pressure higher than 400 MPa (Ohmori et al., 1991). Hardness of beef muscle increases with increase in pressure from 200 to 800 MPa at constant temperature of 20 to 40 °C, but decreases significantly with application of 200 MPa pressure at 60 and 70 °C (Han and Ledward, 2004). The sensory properties vary considerably after high-pressure treatment at 300–700 MPa for 10–20 min. A marked variation is observed in microscopic structure of myofibrils of cattle and mutton muscle. Sarcomere shrinkage occurs with obvious changes. Shear force values of cattle and mutton skeletal muscle reduce markedly (Bai et al., 2004).

Ready-to-eat meats

At the pressure treatment of 600 MPa for 3 min at 20 °C, the counts of aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria* spp., *Staphylococci*, *B. thermosphacta*, *Coliforms*, and fungi are undetectable when stored at 4 °C for 98 days. There is no difference in consumer acceptability and sensory quality of the product (Hayman et al., 2004). The high pressure treatment reduces *L. monocytogenes* by more than 4 log cfu/g in inoculated products.

Fresh raw chicken (minced)

In the pressure range of 400 to 900 MPa for 10 min at 14 to 28 °C, the fresh raw chicken minced sealed polyfilm pouches extend the refrigerated storage life. High pressure processing at 408, 616 and 888 MPa reduces the microbial load by approximately 1.7, 3.4 and 3.7 log cycles, respectively and further storage at 4 °C the microbial spoilage (107 cfu/g) is observed after 27, 70, and >98 days, respectively (O'Brien and Marshall, 1996).

Tenderization of meat

The influence of high pressure on the activity of λ -calpain in pre-rigor meat is markedly reduces during ageing. Total calpain activity decreases, but the activities of acid phosphatase and alkaline phosphatase are not significantly different from those of controls. At pressure range of 100–200 MPa, the inactivation of calpastatin at 100 MPa is faster than that of calpains (Qin et al., 2001).

Prawns and Pork (homogenates)

Shelf-life of prawn is extended up-to 28 and 35 days in samples treated at 200 and 400 MPa, respectively, as compared to 7 days for air-stored samples. The shelf life is extended up to 21 days in vacuum-packaged samples with delayed onset of blackening, whereas high-pressure treatment aggravates the problem (Lopez et al., 2000). At least 6- log cycles reduction in the populations of *E. coli*, *C. jejuni*, *P. aeruginosa*, *S. typhimurium*, *Y. enterocolitica*, *S. cerevisiae*, and *C. utilis* inoculated at level of 10⁶ to 10⁷ cfu/g are observed at high pressure treatment of 400 MPa for 10 min at 25 °C (Shigehisa et al., 1991).

Conclusion

Application of high hydrostatic pressure in food industry for various products is growing day-by-day. From the above discussion, it is quite evident that the high pressure processing technology is versatile in its application and can be utilized for processing of many food products. More over, for commercialization of the high pressure processed products, there is a need of R&D work for process modeling, process validation, study of heat transfer, and measurement of properties of food materials under high pressure processing. Selection of appropriate packaging technology and packaging material is also another concern for viability of this novel technology. Attempts may be made to combine the various non-thermal processes like high pulse electric field, irradiation, magnetic field, osmotic concentration, and application of carbon and argon etc with high hydrostatic pressure processing. At the same time, scientists and processors should keep in mind the safety and quality standards of the high pressure processed foods in order to insure the international quality standards and safety.

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