

## Critical Aspects in Adoption of Ultra High Pressure Technology for Food Processing - An Overviews

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### 식품가공분야에서 초고압 기술의 이용에 대한 고찰

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#### 요 약

신선한 품질의 식품에 대한 수요가 증가함에 따라 열처리의 대체수단으로 이용하기 위한 고압기술의 전망이 밝다. 압력을 이용하여 가공된 과일 주스와 잼이 이미 일본시장에 출시되고 있다. 전세계적으로 다른 식품의 안전을 위해서도 이 기술에 대한 개발노력이 진행중이다. 압력과 열, 시간의 복합효과에 대한 이해는 식품의 안전성에 대한 이 기술의 이용 가능성을 더해준다. 효소의 비활성화와 미생물의 사멸을 위한 압력과 시간의 의존도에 대한 상세한 연구는 식품저장에 초고압기술을 활용하는데 있어 매우 중요하다. 에너지 비용을 절감하기 위한 설계의 개선과 여러 식품성분의 변화에 대한 이해는 이 기술의 응용에 큰 도움이 될 것이다. 안전성과 저장성이 높은 신선한 식품을 생산할 수 있는 초고압 기술은 곧 광범위하게 이용될 전망이다.

Key words: UHP technology, food processing, food safety

### Introduction

In recent years there has been growing concern amongst consumers for safe, additive free and shelf-stable products. The consumer demand for high quality and fresh quality foods, have lead to a new category of foods, called minimally processed foods. Various methods other than the traditional heat treatments have been explored as potential alternatives for minimal processing. These alternative include new ways of applying heat, such as ohmic or microwave heating, and non-thermal methods, such as irradiation, app-

lication of high electric fields, high magnetic fields and high pressure. The hurdle concept or preservation by combined processes, such as a combination of mild heat treatment, addition of preservatives, modification of pH and reduction of water activity and so on is today a common practice in modern food processing to obtain the minimally processed foods<sup>(1,2,3)</sup>.

Presently it is well established that high pressure can be used in food processing to obtain safe foods with identical characteristics to fresh products. This has drawn considerable attention from various food processing industries to explore this technology. High pressure food processing has been explored to prevent growth of microorganisms and to reduce the spoilage caused by the enzymatic

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activities of microorganisms in various foods. Several fruit jams, grapefruit juices, orange juice etc., which have been commercially processed by Ultra high pressure (UHP) are already on sale in Japan. A great deal of interest in this method is also developing in Europe and USA due to consumer demand for high quality foods that are minimally processed, additive free, and microbiologically safe.

UHP technology is considered as an alternative to traditional heating technologies and it also has potential to new products and processes. This also offers an interesting possibilities for food processing ranging from extraction of plant compounds, restructuring of foods and rapid formation of small ice crystals<sup>(3)</sup>. An advantage of this technology is that the food quality characteristics such as flavour and vitamins are unaffected or only minimally altered<sup>(4)</sup>. In addition to food preservation, high-pressure processing opens up the possibilities of producing foods with novel texture<sup>(5)</sup>. High pressure treatments can be used to create new food products with a unique texture or taste with minimum effects on flavour, colour and nutritional value and without any thermal degradation. Hence, this technology has a great potential to produce novel foods with different textural characteristics. The use of hydrostatic pressure foods of high quality, greater safety and increased shelf-life. Food treatments by this method is expected to be less detrimental than conventional processes especially to low molecular weight food compounds such as flavouring agents, pigments, vitamins, etc.<sup>(6)</sup>. In view of the several inbuilt benefits of this technology, attempts have been made from various food sectors to explore this technology for processing a wide range of food products. In view of this growing interest, this article focuses on some of the critical aspects to be considered when adopting this technology for safe and economical food processing.

### General Features of UHP System

The UHP processing of foods is governed by the principle of Le Chatelier. This principle states that phenomena that result in a decrease in volume are enhanced by an increase in pressure and vice versa. According to this, any phenomenon (phase transition, chemical reactivity, change in molecular configuration, chemical reaction) accompanied by a decrease in volume will be enhanced by pressure. One would expect that temperature would have an antagonistic effect because increasing temperature results in a volume increase. On the other hand, reaction rate increase with the increasing temperature according to Arrhenius law. Secondly, in this process, pressure is instantaneously and uniformly transmitted and is independent of the size and the geometry of the food. This is known as isostatic pressure. Any chemical reaction associated with a decrease in total molar volume will be accelerated by pressure. This is combination with mild heat treatment the reaction rate may increase according to Arrhenius law<sup>(7,8)</sup>.

In UHP systems, the pressure is usually generated with a hydraulic pump. The pressurized liquid is enclosed in a steel cylinder of the required thickness and resistance and holding the sample, under pressure for extended period of time does not require extra energy. Industrial machines produced for food treatment operate in a batchwise or in a semi-continuous manner. The system is similar to a conventional heating process wherein the raw material is first transferred into plastic bags, sealed under vacuum, and then pressurized. Liquid products such as fruit juices may be placed directly in the pressure vessel<sup>(1)</sup>. High hydrostatic pressure microbial inactivation depends on a number of interacting factors such as (a) type and number of microorganisms, spore load (b) magnitude and duration of treatment, (c) temperature, (d) composition of the suspension media, besides (e)

the compression and decompression rate. Pressure processing is a function of time and pressure. A low maximum operating pressure can cause drastic reduction in the fabrication cost. Hence it is desirable to combine high pressure processing with moderately high temperatures or other variable so that the operating pressure required could be reduced<sup>(7,9)</sup>. It has been recently reported that the pressure required for microbial inactivation can be reduced if pulsed, successive or oscillatory UHP treatments are applied<sup>(10)</sup>. It was observed that manothermosonication, a combined treatment of heat and ultrasound under moderate pressure was more efficient in inactivating pectinmethylestrase and polygalacturonases than by simple heating process<sup>(11)</sup>. In pressure treatment, temperature change almost always plays a role due to adiabatic heating. In addition there is much interest in the combined effect of temperature and pressure as an effective means of inactivation of microorganisms. Thus, the classical two-dimensional time temperature models have to be replaced by three dimensional time-temperature-pressure models in assessing the effectiveness of UHP in food processing<sup>(3)</sup>. Therefore, it is necessary to evaluate carefully the combination of pressure, time and temperature at which the product has to be processed. The development of high pressure equipment with higher capacity, automated operation, and temperature control is currently underway will help in reducing costs and improving the quality and shelf life foods processed by high hydrostatic pressures<sup>(1)</sup>.

#### Beneficial Aspects of UHP Processing

The growing interest for this technology in food processing is due to its unique benefits over the conventional methods of heating foods. Some of the important advantages are<sup>(1,12,13)</sup>.

(a) Pressure pasteurization is feasible at room temperature and hence energy

- saving as compared to heat treatment.
- (b) Food treated by high pressure pasteurization keeps its original freshness, colour, flavour and taste.
- (c) As the high pressure treatment is a gradient free immediate expansion of pressure which is thus equally effective everywhere in the food.
- (d) Other influence to be expected besides destruction of microorganisms are: protein denaturation or modification, enzyme activation or inactivation, changes in enzyme substrate specificity, changes in the properties of polymer carbohydrates and fats.
- (f) The process has instantaneous effect of pressure throughout the food and the operational at low or ambient temperature so that the food is essentially raw.
- (g) Hydrostatic pressure is energy efficient, and antimicrobial effect is isostatic and instantaneous.
- (h) Since pressure is transmitted instantaneously and homogeneously throughout the food, it does not affect the covalent bonds or produce the over treated zones as may occur in thermal treatments.
- (i) Another added advantage of this process is plastic flexible films could be used and insachet pressure pasteurization could be carried out.

In spite of these benefits the process has a few limitations. Although the vegetative bacteria and fungi are destroyed at lower pressure, the destruction of bacterial spores requires very high pressures. At very high pressures the process may not be economical due to high cost of equipment<sup>(1)</sup>. However, this problem could be minimized by the combination of high pressure with other treatments such as heat. Pressure pasteurization is feasible at room temperature, but the combination of mild heat treatment with high pressure appli-

cation has been shown to be a quite effective technique for food preservation.

#### Sensitivity of Enzymes for UHP Processing

Even small changes in the active site of an enzyme can lead to the loss of enzyme activity. Since the protein denaturation is associated with the conformational changes, UHP may change the functionality of the enzyme. At comparatively low pressure (< 100 MPa), it may stimulate the activity of some of the enzymes. This stimulation effect however is only observed for monomeric enzymes<sup>(8)</sup>. On the other hand much higher pressures generally induce enzyme inactivation. In addition to conformational changes, enzyme activation may arise from pressure induced decompartmentalization. For example, in intact tissues, the enzymes and substrate are often separated by compartmentalization, which can be destroyed upon application of low pressure<sup>(14)</sup>. Pressure induced enzyme damage and the resulting leakage of enzyme and substrate result in enzyme substrate contact. The enzymatic reaction resulting from this contact can, in turn, be accelerated or decelerated by pressure, depending on the reaction volume of the enzyme catalysed reaction.

For pressure induced inactivation of enzymes, there seems to be a minimum pressure requirement, below which no or little enzyme inactivation occurs. When pressures exceeds this value, enzyme inactivation (with a specified time interval) increases until completed at a given pressure. This pressure inactivation range is strongly dependent on various factors such as the type of enzyme, pH, composition of the medium, temperature etc.. It has been reported that the efficiency of enzyme inactivation by high pressure is improved by applying pressure in cycles. Successive application of high pressure resulted in higher inactivation of many enzymes compared to continuous pressure application<sup>(9,10)</sup>. The extent of pressure to be applied depends on the

type, source of enzyme and the environment of food system. Some of the enzymes can be inactivated at room temperature by a few hundred MPa, while others can withstand even above 1000 MPa. Because of the extreme pressure stability of some of the food quality enzymes, combined processes (e.g. pressure and temperature) might be necessary for their inactivation<sup>(15)</sup>.

Besides inactivation of microorganisms, high pressure also induces protein denaturation, enzyme activation or inactivation and changes in enzymes substrate specificity. In the range of 100~300 MPa pressure some of the enzymes may be reversibly inactivated, whereas high pressure can cause irreversible changes. In contrast to this, polyphenoloxidase (PPO) shows an activation followed by inactivation at 500 MPa (10 min, 25°C and pH 6.5). On the other hand pectinmethylesterase (PME) shows no complete inactivation up to 600 MPa (20 min, 20°C, pH 2.5~4.5)<sup>(16)</sup>. The inactivation of PME in citrus juice requires 10 min at 1000 MPa and 20°C or 10 min at 600 MPa and 57°C<sup>(17)</sup>. The same enzyme is much more resistant to pressure at higher sugar concentration (60~65° Brix). For example in fruit juice concentrates a combination of pressure with moderate temperature is needed to inactivate the enzyme. It has been reported that high pressure treatments of about 600 MPa can partially and irreversibly inactivate PME in orange juice<sup>(17)</sup>. Tomato PME seems to be more pressure resistant and its inactivation seems to follow first-order kinetics<sup>(15)</sup>. However, PME of tomato is less pressure stable in the presence of Ca ions or in citric acid buffer (pH 3.4~4.5) than in water, and its pressure stability decreases with decreasing pH. It is interesting to note that at temperature where the enzyme inactivates at atmospheric pressure, an antagonistic effect of pressure and temperature was observed, i.e. inactivation was lower at elevated pressure than at atmospheric pressure<sup>(18)</sup>.

For inactivation of PME a minimum pressure of 800 MPa at 45°C was needed. For PPO more than 30 min with 900 MPa at 45°C was needed. Of all food enzymes tested, PPO, peroxidase (POD) and catalase revealed the highest barostability. Such enzymes therefore serve as indicator enzymes for foods preserved by high pressure treatment. POD is the most heat stable vegetable enzyme. It is also resistant to high pressure. For green beans POD, a treatment of 900 MPa for 10 min at room temperature was needed to cause an 88 % reduction of POD activity. But, when pressure was combined with temperature treatments the inactivation effect enhanced even at 600 MPa. In strawberry puree, POD was increasingly inactivated up to 300 MPa for treatments at 20°C for 15 min. Above 300 MPa, POD activity was further slightly increased. Above 45°C a decrease in activity was found for all pressures (50~400). Highest inactivation rate (50%) was found at 32°C. High pressure treatments at 32~60°C adversely increased POD activity in orange juice<sup>(18)</sup>. Pressure inactivation of soybean Lipoxygenase also follows a first order kinetic model and the kinetic parameters seemed to be strongly dependent on environmental conditions. Pressure resistance of the enzyme increased with increased enzyme concentration and decreased with decreasing pH (pH 9.0~5.4). From the kinetic studies it was observed that the enzymes is most pressure stable around room temperature. Both temperature increase and decrease deviating from the room temperature enhanced the inactivation effect<sup>(8)</sup>. It has been observed with respect to several PPO enzymes that the pressure inactivation proceeds faster at lower pH. In addition to pH, pressure inactivation is also influenced by the addition of salts, sugars or other compounds. The inactivation of PPO in case of avocado at room temperature observed at about 800~900 MPa and there was an antagonistic effect of pressure and temperature at pressure below

250 MPa and temperature exceeding 62.5°C<sup>(19)</sup>.

From the results of some of the above studies it is apparent that laboratory studies can not be directly extrapolated to food system especially for the inactivation of food enzymes. The rate and extent of enzyme inactivation by pressure depends on various factors. The same enzyme behaves in a different way in different environments. Therefore, careful kinetics studies describing the course of inactivation, and pressure and temperature dependence of the inactivation rate constant are of key importance for the design and optimization of combined high pressure/temperature processing for the preservation of food products.

#### Sensitivity of Microorganisms to UHP Processing

The resistance of microorganisms to high pressure is variable. Vegetative cells which are in the growth phase including yeast and moulds, are rather pressure sensitive. Yeast, moulds and most vegetative bacteria including most infections food-borne pathogens can be inactivated by pressure of 300~600 MPa. UHP treatment (500 MPa at 0°C for 10 minutes) was reported to be effective in killing *Vibrio parahaemolyticus*, *Vibrio cholerae* non-01 and *Vibrio mimicus* in sea urchin eggs, while retaining the original flavour and taste<sup>(20)</sup>. UHP causes inactivation of microorganisms and enzymes whereas small molecules such as flavours and vitamins are unaffected<sup>(6)</sup>. It is observed that the vegetative forms of eukaryotes, such as yeast and moulds, are inactivated by pressures between 200 and 300 MPa<sup>(7)</sup>. Gram-positive bacteria are more resistant to heat and pressure than the gram-negative bacteria. *Listeria* and *Staphylococcus aureus*, are more resistant than other gram-positive organisms. The effectiveness of hydrostatic pressure pasteurization on the destruction of several food-borne patho-

gens, namely *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and *Staphylococcus aureus* has been reported<sup>(21)</sup>. *Salmonella senftenberg* 775w. and *Salmonella typhimurium* 7136 were destroyed by pressures ranging from 238 to 340 MPa at 23°C. The rate of destruction of these organisms increased with the increase in pressure<sup>(22)</sup>. Thus it is apparent from some of the above studies that UHP could be used as a potential alternative for heat pasteurization of foods.

Bacterial spores are observed to be highly pressure resistant, as some of the them need pressure exceeding 1,200 MPa for their inactivation. Sterilization of low acid foods (pH < 4.6), on the other hand can most probably rely on a combination of high pressure processing and other mild treatments. For both pasteurization and sterilization processes, combined pressure-temperature treatments are frequently regarded as most appropriate<sup>(8)</sup>. In general, bacterial spores can only be destroyed at very high pressures of more than 1,000 MPa. It has been observed that the pressures above 1,000 MPa are needed to inactivate the spores of *Clostridium sporogenes* in meat broth or carrot broth<sup>(23)</sup>. Heat treatment followed by milder pressures might be more promising for spore destruction. In one of the studies it has been reported that *Clostridium sporogenes* was inactivated at a pressure of 600 MPa for 60 min at 60°C. Heat treatment above 75°C followed by treatment at 800 MPa were also sufficient to inactivate *Clostridium sporogenes* by a factor of at least 105<sup>(24)</sup>. An inactivation ratio of four to six log cycles was obtained for spores of *Bacillus stearothermophilus* when subjected to four to six pressure cycle of 600 MPa at 70°C for 5 min each<sup>(25)</sup>. A further reduction was not noticed when pressure was increased further.

A most heat resistant spore *Bacillus stearothermophilus* was observed to be more sensitive to pressure, whereas a heat sensitive

strain *Bacillus megaterium* was found to be resistant to high pressure. In contrast *Salmonella senftenberg* 775w, a heat resistant strain in more pressure sensitive. Whereas a heat sensitive strain *Salmonella typhimurium* 7136 is more pressure resistant<sup>(22)</sup>. Hence, it can not be generalized that heat resistant microorganisms are also resistant to pressure or *vice versa*. It has been reported that bacterial spores can often be stimulated to germinate by pressure of 50~300 MPa. Germinated spores can then be killed either by mild heat treatment or by mild pressure treatment. However, as such UHP can not be used as an alternative to the sterilization process<sup>(3)</sup> without combination with heat treatment. Viruses are very heterogeneous and the pressure resistance of viruses varies considerably. The number of protein-DNA viruses such as bacteriophages, is considerably reduced at pressures of about 300~400 MPa<sup>(26)</sup>. The number of infectious phage particles could be reduced by at least a factor 106. The spores of yeast and moulds are easily inactivated by pressures of about 400 MPa. Whereas some of the spores such as the ascospores of *Byssoschlamys* are not only resistant to heat but also to pressure treatment<sup>(9,10)</sup>.

It is interesting to note that preservatives such as sorbic acid and nisin are more effective in combination with pressure. Some of the organisms which are normally resistant to nisin can be sensitized to nisin when subjected to pressure treatment. Gram-negative bacteria, such as *E. coli* and *Salmonella* which are normally resistant to nisin, can be sensitized to nisin when pressurized<sup>(23,27)</sup>. Pressurization also inflicts sublethal injury in both Gram-positive and Gram-negative bacterial cells and makes them susceptible to antibacterial compounds, such as bacteriocins and lysozyme<sup>(28)</sup>. It would be possible to obtain a desired level of bacterial destruction within a short time at a moderate hydrostatic pressure along with the application of a mo-

derate temperature increase and suitable antibacterial compounds. Addition of pediocin was also found to increase the sensitivity of bacterial cells to pressures pasteurization<sup>(23)</sup>.

The effects of pressure on microorganisms in food were determined by the temperature during pressure treatment, the food constituents and their properties, and the physiological state of the microorganisms in which the organisms<sup>(3)</sup>. The sensitivity of organisms depends on the environment in which the organisms is present besides the extent of the pressure exerted. The factors such as pH, water activity, ionic strength and time-temperature combination used along with the pressure also play a role in inactivation of microorganisms. Thus, the laboratory studies can not be directly extrapolated to the food system, as the environmental conditions vary significantly. It has been recently observed that bacterial cells are the least sensitive to hydrostatic pressure at 20~25°C but sensitivity increases above 30°C due to phase transition in the membrane<sup>(23)</sup>. In general, bacterial cell death increases as the pressure, time, or temperature increased. However, the cells developed proportionately greater sensitivity as the pressure increased to 276 MPa and the temperature increased to above 35 °C. Pressurization for longer than 5 min, especially at lower temperature and pressure ranges, has a very little added benefit<sup>(23)</sup>. When considering the microbiological safety and stability, the required pressure treatment is dependent on the target microorganisms to be eliminated. Infectious pathogenic bacteria must be completely killed. Toxogenic microorganisms, such as *Clostridium botulinum* and *Staphylococcus aureus* can form toxins in food, but they are harmless as long as they can not grow. Sublethal injury of these microbes is sufficient if combined with suboptimal growth conditions in the food or to extend shelf-life in a peak. The same holds even for spoilage organisms. In all these cases, lethal inactiva-

tion to an acceptably low level guarantees microbiological safety and stability<sup>(3)</sup>.

From a study carried out by Kalchayanand et. al.<sup>(23)</sup> on the pressure inactivation of microorganisms, the following interesting observations have been made: (i) Cell viability loss increased with the increase in pressure and time; (ii) up to 200 MPa, even after 30 min, cell death was < 1 log cycle; (iii) the death rate did not follow first order kinetics, but above 275 MPa the death rate had an initial exponential phase followed by a tailing effect; (iv) Gram negative bacteria showed more sensitivity than Gram-positive bacteria, and in both groups the species and strain differed in sensitivity to pressure; and (v) viability loss was lower in a food system than in phosphate buffer. Although the destruction of bacterial pathogens by hydrostatic pressure is readily attainable, careful kinetic studies are necessary to ensure a desired level of destruction and food safety. This information is important for commercial application of pressure pasteurization of food for microbial destruction of about 7 to 8 log cycles. Similar information will also be important for spoilage bacteria to ensure a long-life for pressure-pasteurized foods. It is necessary to have a combination of high pressure and moderate temperature to obtain microbiologically safe products and economically feasible processes.

#### Mechanism of Microbial Destruction by UHP Process

The effect of UHP processing on microbial destruction and the mode of action varies with the nature and types of microorganisms. High hydrostatic pressure results in protein denaturation, resulting in inhibition of inherent enzymatic activities and the biogenic activity of some microorganisms. It has been reported that inactivation of enzymes seemed to occur more readily than denaturation, indicating that only minor changes in the tertiary structure are responsible for the loss of enzyme activity.

Microorganisms are inactivated by UHP at much lower temperature than the traditional treatments, probably due to pressure induced changes in lipids, enzymes and other essential proteins and soluble constituents<sup>(20, 29)</sup>.

The inactivation of microorganisms when subjected to high pressure is probably due to the destruction of the cell membrane function. It has been proposed that UHP may interfere with the replication of DNA. In one of the studies it has been observed that at pressures of about 100 MPa the nuclear membrane of yeast was affected, and at more than 400~600 MPa further alteration occurred in the mitochondria and the cytoplasm. Above 300 MPa pressure, metal ions have been observed to be released from the cells of microorganisms<sup>(30)</sup>. Elevated hydrostatic pressure can influence gene and protein expression in high pressure adapted microorganisms. Pressure inducible proteins have been found in some of the microorganisms. In case of *E. coli*, pressure of 53 MPa would induce proteins similar to those found at elevated temperature<sup>(3)</sup>. The mechanism might be for the stabilization of structures of membrane-bound enzymes. Exponentially growing cells are more sensitive to pressure than the cells in the stationary phase<sup>(27)</sup>. Pressure inactivation is also accompanied by increase of extracellular ATP, showing the leakage from the cell membrane. UHP can also induce enzyme denaturation. There is some circumstantial evidence that some microbial enzymes constitute the main target of pressure inactivation. Hydrostatic pressure can presumably directly affect the enzymes and carriers of transport system<sup>(31)</sup>. The decrease in intracellular pH, the observation on membrane damage, and protein denaturation during high pressure treatment suggest that membrane-bound enzymes associated with efflux of protons may be at least one of the major targets in high pressure inactivation<sup>(27)</sup>. A further important effect of pressure on membranes would be on ion

movements mediated by ATPase enzymes. DNA and RNA are very resistance to pressure. However, an extreme condensation of the nuclear material was observed in *Listeria monocytogenes* and *Salmonella typhimurium*. The hypothesis is that at elevated pressure, DNA comes in contact with endonucleases, which in turn cleave DNA. This condensation has been found in many other instances and it is reversible and presumably also an enzyme responsible for renaturation is involved. If this enzyme is inactivated by UHP, the cell is no longer able to multiply. As in the case of heat, very severe pressure stress causes a considerable damage to the cell and cells become more sensitive to adverse environmental conditions<sup>(27)</sup>. The bacterial spores are sensitive to pressure between 50 and 300 MPa. At such pressures, spores germinate, followed by death of the germinated spore by pressure application. At pressures over 1,000 MPa, spores are killed more rapidly at low pH values. Germination caused by pressures between 50 and 300 MPa proceeds faster at neutral pH, the net effect is faster destruction, because the germinated spores are susceptible to pressure treatment<sup>(3)</sup>. Though many mechanisms for microbial destruction have been proposed the actual mode of bacterial and enzyme destruction by high pressure treatment is not clearly known.

#### Effect on Textural Characteristics

As it is evident from the foregoing discussion, UHP can destroy microorganisms and enzymes to make food safe for consumption with minimum effect on various valuable constituent of foods. Besides increasing shelf-life and making food safe for consumption, UHP has a great role to play in inducing significant textural characteristics to food materials being processed and aids in production of novel textural food products.

Alteration on the protein conformation by high hydrostatic pressures can bring about

changes in the functional properties of food proteins and hence high pressure treatments of foods can be used to create new products with a unique texture or taste<sup>(32)</sup>. Changes in external factors, such as pressure and temperature, can perturb the subtle balance of intramolecular bonds, therefore, lead to unfolding and denaturation of the polypeptide chain<sup>(6)</sup>. UHP has considerable effects on the non-covalent bonds which are responsible for tertiary structure of proteins, particularly on electrostatic and also on hydrophobic bonds whereas hydrogen bonds are hardly pressure sensitive<sup>(6)</sup>. High pressure (up to 1,000 MPa) can affect protein conformation, aggregation or denaturation, depending on the protein system, the applied pressure, the temperature and the duration of the pressure treatment. At relatively low temperature, covalent bonds are almost unaffected by high pressure, and hence the primary structure will remain intact during pressure treatment<sup>(33)</sup>. Secondary structure changes, on the other hand, occur at a very high pressure and this lead to irreversible denaturation. Significant tertiary structure changes (maintained chiefly by hydrophobic and ionic interactions) are mostly observed in excess of 200 MPa pressure<sup>(34)</sup>. The quaternary structure, held together by non-covalent bonds, are dissociated by comparatively low pressure (<150 MPa). In some cases, pressures exceeding 150 MPa induce reassociation of the dissociated sub units<sup>(35)</sup>. Contrary to thermal treatments, where covalent and non-covalent bonds are affected, high pressure processing at room temperature disrupts relatively weak chemical bonds (hydrogen bonds, hydrophobic bonds, ionic bonds)<sup>(8)</sup>. In general, pressures above 300 MPa cause irreversible changes in protein structure, UHP results in reversible or irreversible changes in the conformation of whey proteins.  $\beta$ -lactoglobulin is more easily denatured than  $\alpha$ -lactalbumin. Specific UHP-induced interactions of whey proteins with

other biopolymers may lead to new protein based food products with novel textures. UHP treatment can be used to induce the denaturation of whey proteins<sup>(36)</sup>. Thus, whey protein gels could be prepared by UHP treatment by selecting appropriate pressure and varying the operating parameters. The strong covalent bonds in protein molecules include peptide bonds and the weaker disulfide bonds. Disulfide bonds play an important role in high pressure induced aggregation and gelation of whey proteins, especially at neutral and alkaline pH values. The increased aggregation might be caused by an increased reactivity of SH groups at such pH values<sup>(6,37)</sup>. The mechanism of denaturation of protein varies with different combination of pressure and temperature used for processing. Thus the texture of food products could be monitored during UHP processing of foods by appropriate selection of processing parameters such as pressure, temperature of the process and the time of exposure besides adjusting the intrinsic factors such as pH, ionic strength etc. of the food system. Hence it has a great role to play in the production of novel-texturized food products.

## Conclusion

With the increasing demand of minimally processed and fresh-like quality foods, there is a great scope for ultra high pressure technology to use as a potential substitute for heat processing of foods. Pressure processed fruit juices and jams have already been launched in Japanese market. Continuous efforts are going on throughout the globe to explore this technology for various other food safety. A better understanding of the combined effect of pressure, temperature and time adds a better image to this technology with respect to food safety and stability. However, a detailed kinetics study with respect to the parameters describing the course of inactiva-

tion, and pressure-temperature dependence of the inactivation rate constant of enzymes and microorganisms is of key importance for design and optimization of combined UHP processing for the preservation of food products. Improvement in design fabrication to reduce the energy requirement and better understanding the behaviour of various constituent of the food system certainly help to adopt this technology with no hesitation. In the coming years UHP is certainly going to be an established technology for food processing as it yields fresh-raw-like product with better safety and stability.

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### References

1. Askar, A. : Minimally processed tropical fruits. *Fruit Processing*, 8, 339 (1998).
2. Jayaprakasha, H. M., Jayaraj, Rao K. and Lokesh Kumar, W. A. : Studies on the influence of water activity (aw) on the stability of foods- A critical appraisal. *J. Food Sci. Technol.*, 34, 273 (1997).
3. Smelt, J. P. P. M. : Recent advances in the microbiology of high pressure processing. *Trends In Food Sci. Technol.*, 9, 152 (1998).
4. Yen, G. C. and Lin, H. T. : Comparision of high pressure treatment and thermal pasteurization effects on the quality and shelf-life of Guava Puree. *Int. J. Food Sci. Technol.*, 31, 205 (1996).
5. Eschtiaghi, M. N., Stute, R. and Knorr, D. : High pressure and freezing pretreatments effects on drying, rehydration, texture and colour of green beans, carrots and potatoes. *J. Food Sci.*, 59, 1168 (1994).
6. Tauscher, B. : Pasteurization of food by hydrostatic high pressure Chemical effects. *Z. Lebensm.-Unters. Forsch.*, 200, 3 (1995).
7. Cheftel, J. C. : Review- High Pressure, microbial inactivation and food preservation. *Food Sci. Technol. Int.*, 1, 75 (1996).
8. Hendrickx, M., Ludikhuyze, M., Van Den Broeck and Weemaes, C. : Effects of high pressure on enzymes related to food quality. *Trends In Food Sci. Technol.*, 9, 197 (1998).
9. Palou, E., Lopez-malo, A., Barbosa-Cano-vas, G. V., Welti-Chanes, J. and Swanson, B. G. : High hydrostatic pressure as a hurdle for *Zygosaccharmyces bailii* inhibition. *J. Food Sci.*, 62, 855 (1998).
10. Palou, E., Lopez-malo, A., Barbosa-Cano-vas, G. V., Welti-Chanes, J. and Swanson, B. G. : Oscillatory high hydrostatic pressure inactivation of *Zygosaccharmyces bailii*. *J. Food Prot.*, 62, 1213 (1998).
11. Lopez, P., Vercet, A., Sanchez, A. C. and Burgers, J. : Inactivation of tomato pectic enzymes by manothemsonication. *Z. Lebensm. Unters. Forsch.*, 207, 249 (1998).
12. Mertens, B. : Development in high pressure food processing. 2. *Lebensm Technol.*, 44, 100 (1993).
13. Ponce, E., Pla, R. Mur-Mur, M., Gervilla, R. and Guamis, B. : Inactivation of *Listeria innocua* inoculated in liquid whole egg by high hydrostatic pressure. *J. Food Prot.*, 61, 119 (1998).
14. Butz, P., Edenharder, R., Fister, H. and Tausscher, B. : The influence of high pressure processing on antimutagenic activities of fruit and vegetable juices. *Food Res. Int.*, 30, 287 (1997).
15. Seyderhelm, I., Boguslawski, S., Michaelis, G. and Knorr, D. : Pressure induced inactivation of selected food enzymes. *J. Food Sci.*, 61, 308 (1996).
16. Takahashi, Y., Ohta, H., Yonei, H. and

- Ifuku, Y.: Microbiocidal effect of hydrostatic pressure on satsuma mandarin juice. *Int. J. Food Sci.*, 28, 95 (1993).
17. Ogawa, H., Ffukuhisha, K., Kubo, Y. and Fukumoto, H. : Pressure Inactivation of yeasts, Molds and Pectinest erase in Satsuma Mandarin juice. Effects of juice concentration, pH and organic acids, and comparison with heat sanitation. *Agric. Biol. Chem.*, 54, 1219 (1990).
  18. Cano, M. P., Hernandez, A. and De Ancos, B. : High pressure and Temperature. Effects in Enzymes Inactivation in strawberry and orange products. *J. Food Sci.*, 62, 85 (1997).
  19. Weemaes, C. A., De Cordt, S. V., Ludikhyyze, L. R., Van Den Broeck, I., Hendrickx, M. E. and Tobback, P. P. : Influence of pH, Benzoic acid, EDTA and Glutathione on the pressure and/or Temperature Inactivation Kinetics of mushroom polyphenoloxidase. *Biotechnol. Progr.*, 13, 25 (1997).
  20. Ohshima, T., Ushio, H. and Koizumi, C. : High pressure processing of fish and fish products. *Trends In Food Sci. Technol.*, 4, 370 (1993).
  21. Patterson, M. F., Quinn, V., Simpson, R. and Gilmore, V.: Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. *J. Food Prot.*, 58, 524 (1995).
  22. Metrick, C., Hoover, D. G. and Farkas, D. F. : Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of Salmonella. *J. Food Sci.*, 54, 1547 (1989).
  23. Kalchayanand, N., Sikes, T., Dunne, C. P. and Ray: Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Appl. Environ. Microbiol.*, 60, 4174 (1994).
  24. Crawford, Y. J., Murano, E. A., Olson, D. G. and Shenoy, K.: Use of high hydrostatic pressure and irradiation to eliminate the *Clostridium sporegenes* spores in chicken breast. *J. Food Prot.*, 59, 711 (1996).
  25. Hayakawa, K., Kanno, T., Tomito, M. and Fujio, Y.: Oscillatory compared with continuous high pressure sterilization. *J. Food Sci.*, 69, 164 (1994).
  26. Brauch, G., Haensler, V. and Ludwig, H. : The effect of pressure on Bacteriophages. *High Pressure Res.*, 5, 767 (1990).
  27. Ackey, B. M., Forestiere, K. and Issacs, N. : Factors affecting the resistance of *Listeria monocytogenes* to high hydrostatic pressure. *Food Biotechnol.*, 9, 363 (1995).
  28. Hauben, K. J. A., Wuytack, E. Y. Soontjens, C. F. and Michiels, C. W. : High pressure transient sensitization of *Escherichia coli* to lysozymes and nisin by disruption of outer membrane permeability. *J. Food Prot.*, 59, 350 (1996).
  29. Ludikhuyze, Indrawati, I. Van Den Braeck, C. A. Weemaes and Hendrickx, M. E.: High pressure and thermal denaturation kinetics of soybean Lipoxygenase. A study based on Gel Electrophoresis. *Lebensmittel-Wissenschaft Und Technologie.*, 31, 680 (1998).
  30. Shimada, S., Andou, M., Naito, N., Osumi, M. and Hayashi, R.: Effects of hydrostatic pressure on the ultra structure of internal substances in the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.*, 123 (1993).
  31. Jaenicke, R. : Protein stability and molecular adaptation to extreme conditions. *Eur. J. Biochem.*, 202, 715 (1991).
  32. Messens, W., Van Camp, J. and Huyghebart, A. : The use of high pressure to modify the functionality of the food proteins. *Trends In Food Sci. Technol.*, 8, 107 (1997).
  33. Hayashi, R. : Application of high pressure to food processing and preservation. Philosophy and Development in Engineering and Food (Spiess, W. E. L. and Schubert, eds). *Applied Science, England.* 81 (1989).

34. Balny, C. and Masson, P. : Effects of high pressure on proteins. *Food Rev. Int.*, 9, 611 (1993).
35. Ohmiya, K., Kajino, T., Shimijo, S. and Gekko, K. : Effect of pressure on the association site of enzyme treated caseins. *Agric. Biol. Chem.*, 53, 1 (1989).
36. Kessler, H. G. : Whey protein denaturation, aggregation and application. Proc. 25th International Dairy Congress, 21-24 September, 28 (1998)
37. Funtenberger, S., Dumay, E. and Cheftel, J. C. : Pressure induced aggregation of  $\beta$ -lactoglobulin in pH 7.0 buffers. *Lebensm.-Wiss. Technol.*, 28, 410 (1995).
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