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REVIEW

Effect of High Pressure Treatment on Organoleptic and Microbial Quality of Food Products

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In recent years high pressure processing has been investigated as an alternative method for food preservation. High pressure technology allows inactivation of microorganisms while maintaining sensory and nutritional properties of foods. The present paper gives an overview of approaches which have been reported in literature about inactivation vegetative bacterial cells, bacterial spores, viruses, algal biotoxin, yeast and moulds, as well as effect of high pressure treatment on colour, flavour and texture of food products.

Key Words: High pressure treatment, Inactivation, Colour, Flavour, Texture.

INTRODUCTION

Thermal food preservation is a well known and old technique for reducing the microbial count of foods For heat sensitive food products, however, thermal pasteurization can impart undesirable organoleptic changes in addition to some detrimental affects to the nutritional quality of the food. Consumers require safe food, but increasingly they also demand minimally processed, additive-free foods with an extended shelf-life.

Physical treatment methods such as pulsed electric fields, ultra-violet radiation, oscillatory magnetic fields and high pressure processing all have the potential to destroy food-borne microorganisms without much affecting the quality of food products¹.

The characteristics of an ideal processing method have been identified² as: (a) able to inactivate spoilage and pathogenic microorganisms, (b) not degrading organoleptic and nutritional values of products, (c) not leaving residues, (d) cheap and convenient to apply and (e) acceptable to consumers and regulatory agencies.

Pressure is a fundamental thermodynamic quantity, comparable to temperature or chemical potential. This reveals in turn pressure as a 'basic tool for research, specific structure creation and process design'³⁻¹⁵. High pressure-processed foods were first commercialized in Japan in 1992. Following initial successes with fruit juices and jams, the technology has been applied to an increasing range of food products, including smoothies, ham, guacamole, salsa, rice products, fish and shellfish.

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In some researchs to find ideal processing characteristics two or more processing methods are commonly applied simultaneously. Combinations of treatment are often more effective at preventing microbial growth than those same conditions used in isolation, which means combining preservative factors can significantly improve the quality of foods whilst delivering the same level of microbial inactivation as conventional methods. Such a combination using high temperature applied with high hydrostatic pressure can successfully inactivate microorganisms.

Colour, flavour and texture are important quality characteristics of fruits and vegetables and major factors affecting sensory perception and consumer acceptance of foods. High pressure processing could preserve nutritional value¹⁶ and the delicate sensory properties of fruits and vegetables due to its limited effect on the covalent bonds of low molecular-mass compounds such as colour and flavour compounds. However, food is a complex system and the compounds responsible for sensory properties coexist with enzymes, metal ions, *etc.*

This review discusses the current findings on how high pressure processing affects the the organoleptic quality of foods and the ability of high-pressure treatment for inactivation of bacteria.

Effect of high pressure treatment on colour: High pressure treatment (at low and moderate temperatures) has a limited effect on chlorophyll, carotenoids, anthocyanins, *etc*.

Chlorophyll is a green compound found in the leaves and green stems of plants. Chlorophylls a and b have different stabilities towards pressure and temperature. At room temperature, chlorophylls a and b exhibit extreme pressure stability but at temperatures higher than 50 °C, high pressure treatment affects their stability for example, a significant reduction in the chlorophyll content of broccoli juice^{17,18}. At a constant pressure level, the values of the degradation rate constants of chlorophylls increase with increasing temperature¹⁸ whereas at constant elevated temperatures, pressure increase accelerates the degradation of chlorophyll b at 70 °C is higher than that of chlorophyll a. For example, elevating pressure from 200 to 800 MPa accelerates the degradation of chlorophyll b of broccoli by 19.4 and 68.4 %, respectively¹⁸. Matser *et al.*¹⁹ also reported chlorophyll degradation of green beans and spinach due to high pressure processing at elevated temperatures, even for a short exposure time (two pulses of 90 °C/700 MPa/1 min).

High pressure treatment at ambient and moderate temperatures results in limited colour change of green vegetables. However, at elevated temperature, the green colour shifted visibly to olive-green with a concomitant increase in the a* value for example, green beans after high pressure treatment at elevated temperature (two pulses of 1000 MPa/75 °C/80 s)²⁰ or basils after high pressure treatment of 860 MPa/75 °C/80 s or 700 MPa/85 °C/80 s²¹.

Carotenoids are responsible for the orange-yellow and red appearance of fruits and vegetables. Carotenoids are rather pressure stable. High pressure treatment increases the extraction yields of carotenes from the plant matrix²⁰⁻²⁴. The colour of tomato pure remained unchanged after high pressure treatment (up to 700 MPa) at 65 °C even for 1 h^{25} .

Anthocyanins are water-soluble vacuolar flavonoid pigments responsible for the red to blue colour of fruits and vegetables. Anthocyanins are stable during high pressure treatment at moderate temperature, for example, pelargonidin-3-glucoside and pelargonidin-3-rutinoside in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria x ananassa*) during high pressure treatment at 800 MPa (18-22 °C/15 min)²⁶.

Besides the instability of colour pigments, browning plays an important role in the decolouration of high pressure-treated food products. In fruit-based food products, no visual colour differences (based on L*, a* and b* values) are observed immediately after high pressure treatments, for example in white grape juice after high pressure treatment at 400 MPa/2 °C, 500 MPa/2 °C or 400 MPa/40 °C/10 min²⁷ or in mango pulps after high pressure treatments at 100-400 MPa/20 °C/15 or 30 min²⁸. Ahmed *et al.*²⁸ observed that colour parameters such as (a/b), C and h values of mango pulps remained constant after high pressure treatment indicating pigment stability, while increasing pressure intensity decreased the value of DE. Changes in colour appearance would be more expected rather than the changes in pigment concentration²⁹. Colour changes in high pressure -treated fruits and vegetables can be related to changes in textural properties. This phenomenon was observed in tomato based products.

Effect of high pressure treatment on texture: Texture changes in fruits and vegetables can be related to transformations in cell wall polymers due to enzymatic and non-enzymatic reactions³⁰. Due to cell disruption, high pressure processing facilitates the occurrence of enzymatic and non-enzymatic reactions. Substrates, ions and enzymes which are located in different compartments in the cells can be liberated and interact with each other during high pressure treatment. At the same time, pressure can enhance the action of pectinmethylesterase (PME), lower the polygalacturonase (PG) activity (occurring mostly at moderate temperature) and retard β -elimination [a reaction where loss of two substituents from adjacent atoms (such as carbon, nitrogen, oxygen) results in the formation of new unsaturated bond] (possibly occurred at elevated temperatures). Pectinases, such as orange PME³¹, strawberry PME³², tomato PG³³, carrot PME³⁴, banana PME³⁵, pepper PME³⁶ and plum PME³⁷ show differences in their pressure and temperature stability.

Basak and Ramaswamy³⁸ studied the effect of high pressure processing (100-400 MPa/5-60 min/room temperature) on the firmness of different fruits and vegetables such as apple, pear, orange, pineapple, carrot, celery, green pepper and red pepper. The authors observed a rapid firmness loss during compression. During the pressure holding period (30-60 min), the firmness either decreased further or recovered gradually, such as for pear, orange, pineapple, carrot, celery, green pepper and red pepper treated at 100 and 200 MPa. Pectinmethylesterase activity was suggested to be the

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major reason for the observed increase in firmness. Upon high pressure treatment, pectinmethylesterase is liberated and contacts its substrate, the highly methylated pectin, leading to demethylation.

High pressure treatment can affect the rheological properties of food products such as crushed fruits and vegetables, pure, pulp and juice. The observed effects are dependent on the conditions of the high pressure process and the type of fruit and vegetable. Ahmed *et al.*²⁸ reported that the viscosity of mango pulp increased after high pressure treatments at 100 or 200 MPa (20 °C/15 or 30 min), while a reduction in viscosity was observed after high pressure treatments at 300 and 400 MPa (20 °C/15 or 30 min). The viscosity of tomato homogenate decreased considerably at pressures 400 MPa but increased at higher pressure levels, such as 500 MPa combined with temperatures up to 60 °C^{39,40}. However, in the presence of NaCl (0.8 %), the effect of pressure was the opposite. The viscosity increased with increasing pressure up to 400 MPa³⁹.

In relation to cheese texture and microstructure, Buffa *et al.*⁴¹ using uniaxial compression and stress relaxation tests and confocal laser scanning microscopy showed that cheese made from raw or high pressure-treated milk were firmer and less fracturable than cheese made from pasteurized milk, but differences became less notable toward the end of ripening. Cheese from pasteurized and high pressure-treated milk were less cohesive than from raw milk. Although cheese exhibited a loss of elastic characteristics with ageing, cheeses from high pressure-treated milk were the most elastic initially. Confocal laser scanning micrographs displayed cheese from high pressure-treated milk with a regular and compact protein matrix, with small and uniform fat globules resembling the structure of cheese made from raw milk. These findings show that high pressure processing of milk could be an alternative to heat treatment for the production of fresh or ripened cheese with improved performances.

The properties of acid-set gels prepared from high pressure-treated milk have been reported by Johnston *et al.*⁴². Results indicate improved texture (rigidity and resistance to breaking) and syneresis resistance of the gels, measured by drainage or by centrifugation. Furthermore, authors reported viscosity improvement of stirred-style yoghurt-type product prepared from high pressure-treated skim milk (100-600 MPa, up to 1 h). Most of the viscosity improvement was achieved after 15 min pressurization at 400 MPa and after 5 min at 600 MPa with slight further increases up to 1 h.

Ferragut *et al.*⁴³ elaborated ewe's milk yoghurt from high pressure-treated milk using different combinations of temperature and pressure (10, 25 and 55 °C; 200, 350 and 500 MPa for 15 min) and from pasteurized (70 °C, 10 min) milk. Yoghurt firmness increased as pressure increased and treatments of 350 MPa at 25 °C and 500 MPa at 55 °C showed no, differences in whey syneresis compared with pasteurized milk. Yoghurt evolution in storage at 4 °C for 20 d showed a good stability in terms of firmness in all treatments but water retention was only maintained in yoghurts made from high pressure-treated milk.

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Whipping properties improved when cream was treated at pressures up to 600 MPa for up to 2 min⁴⁴ probably due to better crystallization of milk fat. When treatment conditions exceed the optimum an excessive denaturation of whey protein occurs and results in longer whipping time and destabilization of whipped cream. Below 400 MPa no noticeable effects on whipping properties of cream were found.

Effect of high pressure treatment on flavour: It is generally assumed that the fresh flavour of fruits and vegetables is not altered by high-pressure processing, since the structure of small molecular flavour compounds is not directly affected by high pressure. This has been observed, by means of both chemical and sensory analysis, in a number of studies where strawberry pure⁴⁵, mandarin juice⁴⁶, orangeelemonecarrot juice²², white grape juice²⁷ and guava juice⁴⁷ have been treated at pressures of 200-600 MPa combined with ambient temperature. As high pressure processing can enhance and retard enzymatic and chemical reactions, it could indirectly alter the content of some flavour compounds and disturb the whole balance of flavour composition in fruits and vegetables. As a consequence, high pressure processing could result in undesired changes in flavour. Hexanal is a volatile compound associated with the smell of foliage and grass. Gas chromatographic studies showed changes in the hexanal content of fruits and vegetables as a result of high pressure processing. Navarro et al.48 observed that high pressure processing at 400 MPa (ambient temperature/20 min) more than doubled the hexanal content of strawberry pure. Lambert et al.⁴⁵, on the contrary, observed less pronounced effects of pressure on the hexanal content of strawberry pure but pressurization at 800 MPa (ambient temperature/20 min) resulted in a slight decrease in the hexanal content.

Porretta *et al.*⁴⁹ reported that high pressure treatment (500, 700 or 900 MPa/ room temperature/3, 6 or 9 min) of fresh tomato juice resulted in the generation of such a strong rancid taste, that the juice was unsuitable for sensory analysis. *n*-Hexanal was suggested to be responsible for the rancid taste, because the *n*-hexanal content in all pressure-treated tomato juices was much higher (6.4 mg kg⁻¹) than in the fresh juice (0.3 mg kg⁻¹).

Regarding strawberry based food products, high pressure processing at 800 MPa (20 $^{\circ}C/20$ min) modified the flavour profile of pure strawberry⁴⁵.

Ester compounds belong to the most important flavour compounds in strawberries but the stability of ester compounds during pressure is still under discussion. Lambadarios and Zabetakis⁵⁰ observed only a small decrease in ester concentration when model systems containing fruit esters in buffer solution were subjected to high pressure treatment (400 or 800 MPa/18-22 °C/15 min) at various pH values (pH 4, 6 and 8). Lambert *et al.*⁴⁵ also reported the presence of many esters in high pressure treated (200, 500 or 800 MPa/20 °C/20 min) strawberry pure. Zabetakis *et al.*⁵¹ on the contrary, found no ester compounds in high pressure (200, 400, 600 or 800 MPa/18-22 °C/15 min) treated strawberries. It is possible that the ester compounds in the study of Zabetakis *et al.*⁵¹ were lost during sample extraction. Gimenez *et al.*⁵² reported that strawberry jam prepared by high pressure processing (400 or 800

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MPa/22 °C/5 min) smelled more chemical, rancid and less fruity than traditionally processed jam. However, none of the flavour compounds generated by heat-sterilization (120 °C/20 min) was found in high pressure-treated (200-800 MPa/ambient temperature/20 min) pure strawberry⁴⁵.

Inactivation of bacteria by high pressure treatment: High pressure can inactivate microorganisms, high pressure can damage membranes, denature enzymes and cause changes in cell morphology^{53,54}. Hoover *et al.*⁵³ proposed that in a similar way to thermal inactivation, high pressure does not disable one specific site, but acts on a variety of targets depending on the applied pressure. Cell membranes are thought to be a primary target for high pressure inactivation of bacteria^{1,55} and evidence for this is provided by the relationship between pressure resistance and membrane fluidity⁵⁶. Furthermore, it has been suggested that susceptibility to high pressure of Gram-negative bacteria compared to Gram-positive bacteria is due to the complexity of Gram-negative bacteria cell membranes⁵⁷. High pressure disrupts membrane function and causes leakage through the inner and outer membranes, as demonstrated for high pressure -treated cells by their increased sensitivity to sodium chloride and bile salts⁵⁸, uptake of propidium iodide and ethidium bromide⁵⁹ and leakage of ATP⁶⁰. Membrane perturbation is attributed to the promotion of phase transitions in the phospholipid bilayer from liquid to more tightly packed gel phases. High pressure can also denature or displace membrane-bound enzymes. For example, the activity of F0F1 ATPase in Lactobacillus plantarum was reduced following high pressure treatment, impairing the cells ability to efflux protons and regulate their internal pH. The inactivation or disruption of key enzymes by high pressure can lead to microbial inactivation and similar patterns in enzyme denaturation and inactivation of microorganisms have been reported^{56,61}.

In addition, high pressure induces changes in morphology and internal organization of cells, including cell lengthening, contraction of the cell wall and pore formation, separation of the cell membrane from the cell wall and compression of gas vacuoles⁵⁵. Altered distributions of DNA and ribosomes⁵⁸ and ribosome destruction⁶¹ have also been observed in high pressure-treated cells and a correlation between cell death and ribosome damage has been suggested⁶².

Many studies have shown that pressures in the range of 300-600 MPa can inactivate many fungi and vegetative bacteria¹; however microorganisms can differ widely in their intrinsic susceptibility to high pressure. Bacteria, in particular, demonstrate a wide range of resistance to high pressure. Gram-negative bacteria tend to be more sensitive to high pressure than Gram-positive species^{1,63}, but there are many exceptions to this generalization, for example, certain strains of *E. coli* O157 are exceptionally pressure resistant.

Bacterial spores are very resistant to inactivation by high pressure. The spores of *Clostridium botulinum* strains can survive extreme treatment conditions (827 MPa for 30 min at 75 $^{\circ}$ C)⁶³. However, the use of oscillatory high pressure treatments, where a lower high pressure induces spores to germinate, allowing their inactivation

by a subsequent cycle at a higher high pressure, has proved successful^{64,65}. Vegetative forms of yeasts and moulds are the most pressure sensitive¹. Yeasts and moulds are relatively sensitive to high pressure. However, the ascospores of some fungi show a pressure resistance that is comparable to that of the most resistant bacterial cells. For instance, inactivation of *Byssochlamys nivea* ascospores requires a pressure temperature treatment above 600 MPa and 60 °C⁶⁶. Furthermore, high pressure treatment can induce germination of dormant fungal spores, for instance of *Talaromyces macrosporus*⁶⁷.

Several works have been done for studying the effect of high pressure on inoculated target microorganisms in ewe's milk, with the aim of determining the sensitivity of pathogenic and spoilage microorganisms in milk. In general, the high pressure inactivation was greater on *P. fluorescens*, *E. coli*, *L. innocua*, *L. helveticus* and *S. aureus*. The temperature effect in addition to the high pressure on microorganisms was different. The *P. fluorescens*, *L. innocua* and *L. helveticus* showed higher resistance to high pressure at room temperature (25 °C) than at low temperature (4 °C), whereas *E. coli* and *S. aureus* showed less resistance to high pressure at room temperature than at low temperature⁶⁸.

High pressure technology can be used to increase the microbiological safety and quality of milk to produce high quality cheese, Most research to date has concentrated on the application of high pressure to inactivate microorganisms in cheese to increase cheese safety and shelf life. Szczawinski *et al.*⁶⁹ achieved a 6 log reduction of inoculated *Listeria monocytogenes* in ripened sliced cheese with a treatment of 500 MPa for 15 min and a significant decrease of cheese microbiota. Gallot-Lavalle⁷⁰ studied the efficiency of high pressure treatment for destruction of *L. monocytogenes* in goat cheese from raw milk finding that 450 MPa/10 min or 500 MPa/5 min treatments achieve more than 5.6 log units of reduction of this microorganism without significantly affecting sensory characteristics of cheese. Reps *et al.*⁷¹ achieved a significant decrease of total microbial counts at pressure above 400 MPa in Gouda and Camembert cheeses, whereas spore count was unaffected even at 1000 MPa.

Capellas *et al.*⁷² observed reductions of 7 log units of *Escherichia coli* populations working on inoculated Mato-cheese (fresh goat's milk cheese) and high pressuretreated from 400 to 500 MPa for 5-15 min at refrigeration and room temperature and the extension of refrigerated storage life of the cheese. These authors also studied the resistance of cocci (*Staphylococcus carnosus*) and spores (*Bacillus subtilis*) in fresh cheese as these groups of microorganisms are acknowledged as pressure resistant. The treatments that provided a total inactivation of *E. coli* only reduced *S. carnosus* population in 2 log units.

O'Reilly *et al.*⁷³ have determined the effect of high pressure (50-800 MPa for 20 min at 10-30 °C) on the inactivacion of microbial contaminants (*Staphylococcus aureus, E. coli* and *Penicillium roqueforti* spores) in model cheese systems (phosphate buffer at pH 5.3 and cheese slurries) and in Cheddar cheese. Relative sensitivity of the microbial species to high pressure in Cheddar cheese was, as it was demonstrated previously in model cheese slurry system, *P. roqueforti, E. coli, S. aureus.* How-

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ever, pressure inactivation of *S. aureus* and *P. roqueforti* was most extensive in buffer while, a greatest sensitivity was exhibited by *E. coli* in Cheddar cheese at pressures -200 MPa, possibly due to acid injury during the cheese fermentation.

The application of high pressure for kefir preservation has also been studied. Reps *et al.*⁷⁴ studied the microbial populations and acidifying activity of kefir treated at 200-800 MPa during 15 min and stored for 3 weeks. Reduction of bacterial counts increased with increasing pressures and yeasts were completely inactivated at 400 MPa. Acidification of kefir pressurized at 600 and 800 MPa only increased slightly during the storage. Mainville *et al.*⁷⁵ have also studied the deactivation of bacteria and yeast in kefir using heat treatment, irradiation and high pressure. Heat treatments (autoclaving at 110 °C for 3 min and ohmic heating at 72 °C internal temperature) deactivated the bacteria and yeast in kefir (8.58 log cfu *lactobacilli* and 5.09 log cfu total yeasts) but changes in structure of the kefir protein and lipids were seen in transmission electron micrographs. Irradiation of kefir at 5 kGy and high pressure treatment at 400 MPa for 5 or 30 min deactivated the bacteria and yeast in kefir and left the protein and lipid structure of the product unchanged.

The studies carried out by Raffalli *et al.*⁷⁶ have shown that it is possible to reduce significantly the microbial load of a dairy cream (35 % fat) by high pressure at 450 MPa and 25 °C for 10 to 30 min. Inactivation followed apparent first order kinetics, with a decimal reduction time of 7.4 min under the pressure treatment conditions used.

Inactivation of viruses by high pressure treatment: Viruses are a structurally diverse group of organisms that also differ widely in their sensitivities to high pressure. For example, feline calicivirus (a norovirus surrogate) is inactivated by treatment at 275 MPa for 5 min⁷⁷. In contrast, poliovirus is very resistant to high pressure, with no significant reductions in infectivity reported after relatively severe, treatments, such as 600 MPa at 20 °C for 1 h⁷⁸. The reason for the disparate resistance of viruses to high pressure is not known. It has been suggested that the resistance of poliovirus may be related to the size and shape of the virus particle⁷⁸ or its high thermodynamic stability⁷⁹.

The high pressure-inactivation of bacteriophages T4 and E is due to the displacement of their DNA and the formation of empty protein shells⁸⁰. In contrast, no structural changes in high pressure-inactivated AX phage were determined and the authors suggest that AX phage reassociates following high pressure treatment, without the restoration of infectivity. Similarly, the loss of infectivity of other viruses following high pressure treatment has been attributed to relatively subtle modifications⁸¹.

Effects of high pressure on algal biotoxins: Six recognized human poisoning syndromes from algal biotoxins (paralytic, neurotic, amnesic, diarrhetic shellfish poisonings, ciguatera fish poisoning and putative estuary, associated syndrome) impact on human health through consumption of contaminated seafood, contact with bloom water or inhalation of aerosolized toxin⁸². Although there are no studies on the effects of high pressure on algal toxins to date, their lack of secondary, tertiary and quaternary structure implies that high pressure may not affect toxins.

Conclusion

High pressure processing is a unique technology compared to other food processing technologies. The effects of high pressure on both microorganisms and organoleptic quality are highly dependent on processing parameters and the complex nature of high pressure necessitates the careful design of processing regimes to maximize the goal of microbial inactivation, while maintaining optimal product quality. However, high pressure processing offers many advantages over conventional processing methods. the effect of high pressure treatment on sensory properties cannot be generalized since (i) study on basic insight in this subject is still limited and (ii) sensory property is product dependent.

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