Novel approaches in food-processing technology: new technologies for preserving foods and modifying function

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Recent advances in emerging food-processing technologies, such as high hydrostatic pressure or high-intensity electric field pulses, allow targeted and sophisticated modification and preservation of foods. We are beginning to understand the mechanisms involved in pressure inactivation of bacterial spores and have been collecting considerable amounts of kinetic data regarding inactivation mechanisms of enzymes and vegetative microorganisms. We are also gaining more insight into the permeabilization of plant membranes and related biosynthetic responses, making progress in food structure engineering and food modification for function, and have been initiating process developments for gentle processing of delicate biomaterials based on pressure-assisted phase transitions of water.

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Abbreviations
HELP high-intensity electric field pulse
HP high pressure

Introduction

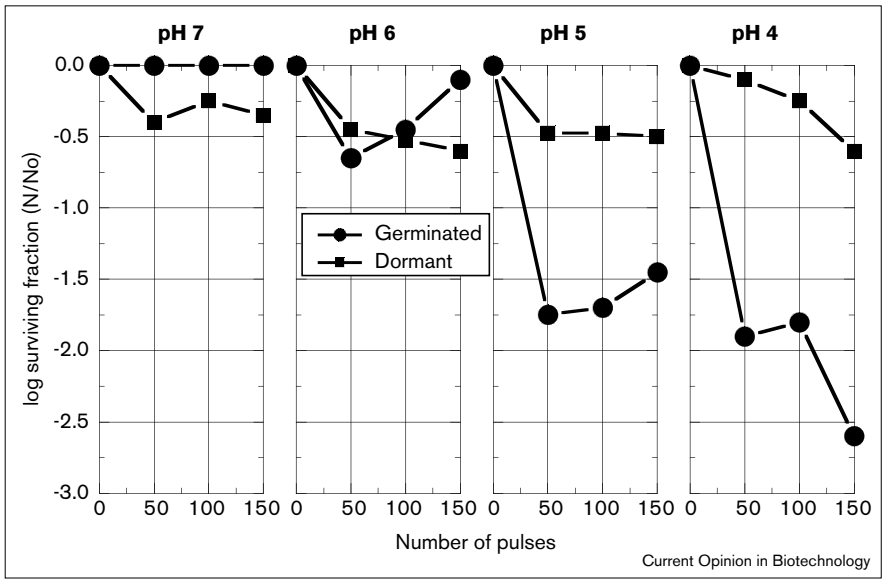
The main advantages of the emerging food technologies to be discussed in this review, such as high pressure (HP) and high-intensity electric fields include instant distribution of the active principle (e.g. pressure) throughout the samples and consequent independence of size and geometry. This poses a significant advantage over conventional thermal processes where the dependence of size and geometry for mass and heat transfer are critical process variables and limitations. These technologies offer low temperature applications leading to quality enhancements and energy efficiency and are practically waste-free technologies. Quality and function retention and improvement is also made possible by either short processing times (i.e. high-intensity electric field pulses [HELPs]) or by limiting processing effects mainly to impacts on non-covalent bonds (i.e. high hydrostatic pressure).

Reaction enhancement (i.e. enzyme activities) can be obtained under elevated pressures. By adding pressure (p) to the conventionally applied time (t) and temperature (T) matrix for food processing, better targeted and sophisticated processes ($p \times t \times T$) are now possible.

High-intensity electric field pulse treatment

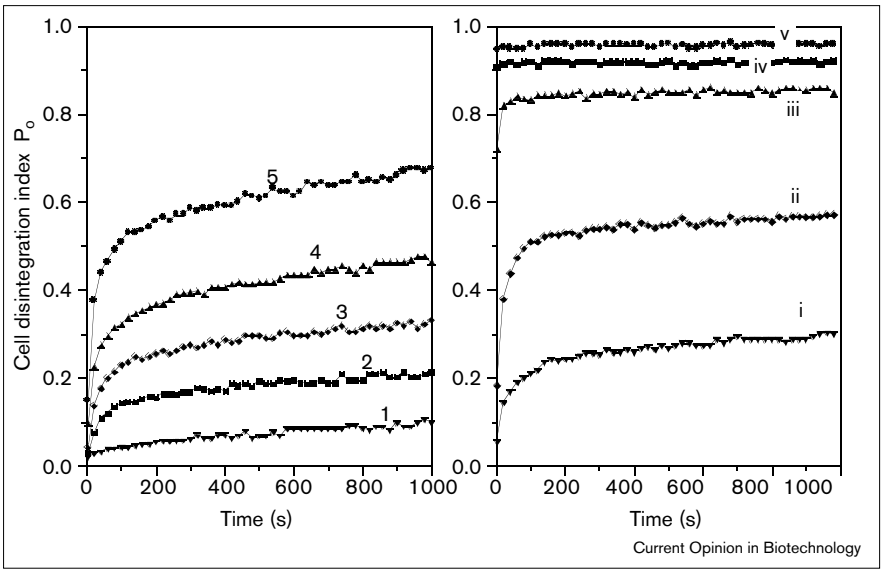
Treatment of food systems with HELPs is a reemerging technology after previous attempts at application in the early 1960s, and concentrates mainly on the inactivation of microorganisms [1•]. However, the irreversible permeabilization of plant membranes to improve mass transfer of metabolites has also become a focal point of recent attention [2•]. Engineering aspects of HELP equipment and process design, especially treatment chamber development and process homogeneity, are also receiving increasing attention [3•].

Figure 1



Induction of germination of *Bacillus subtilis* spores by HELP treatment in conjunction with low pH (based on Heinz *et al*., personal communication). Inactivation of germination spores after HELP was obtained by thermal treatment (70°C for10 min).

Figure 2



The kinetics of the cell permeabilization after application of single pulse on potato cell cultures **(left)** with various energy input and **(right)** after 1–20 pulses with constant energy input per pulse of 0.6 kJ/kg. Energy input (kJ/kg): 1 = 0.07; 2 = 0.17; 3 = 0.6; 4 = 1.7; 5 = 5.6. Number of pulse: i = 1; ii = 2; iii = 5; iv = 10; v = 20. The first measurement for p_o calculation was taken 2 s after the pulse application.

Field strengths applied for plant membrane permeabilization are usually between 0.5 to 5.0 kV/cm, whereas for distruction of microbial membranes a field strength above 15 kV/cm is required. Pulse durations are in the microsecond to millisecond range and the most generally applied pulse geometries are exponential decay, which are currently being evaluated and optimized. Treatment chamber designs for food application include parallel plate, coaxial or co-linear configurations. Prototype pilot-scale equipment with capacities of 300 L/h (commercial) and 500 kg/h (our laboratory) is available. Currently, no commercial HELP process for food processing exists.

Membrane permeabilization

Inactivation of vegetative microorganisms by HELP, as well as resistance of bacterial spores to HELP treatment has been reported [4]. Interestingly and unexpectedly, germination of spores could be induced in low pH environments by HELP (Figure 1).

To effectively quantify the impact of HELP on plant membrane disintegration, a quantifiable measure was required. Based on a study of the electrophysical behaviour of intact cells with insulated biological membranes, a model of a cell system also with different ratios of intact cells, ruptured cells and extracellular compartments was developed [5•,6••]. Based on the relative changes of sample conductivities, which were obtained within a characteristic low and high frequency range of the characteristic β-dispersion (interfacial electrical polarization effector), a measure of cell permeabilization, the cell disintegration index (p_o), was established:

$$p_o = \frac{\frac{\sigma^i_h}{\sigma^t_h} \cdot \sigma^t_l - \sigma^i_l}{\sigma^i_h - \sigma^i_l}$$

Determination of p_o in a processed cell system is carried out by measuring the conductivity of initial intact (σ_l^i and σ_h^i) and of treated (σ_l^t and σ_h^t) samples at low and high frequencies within the band of β-dispersion (for most plant and animal cells in suspension culture or tissue the characteristic low frequency range is in order of 10^3 Hz and high frequency range in the order of 10^7 Hz). For intact cells, $p_o = 0$; for total cell disintegration, $p_o = 1$.

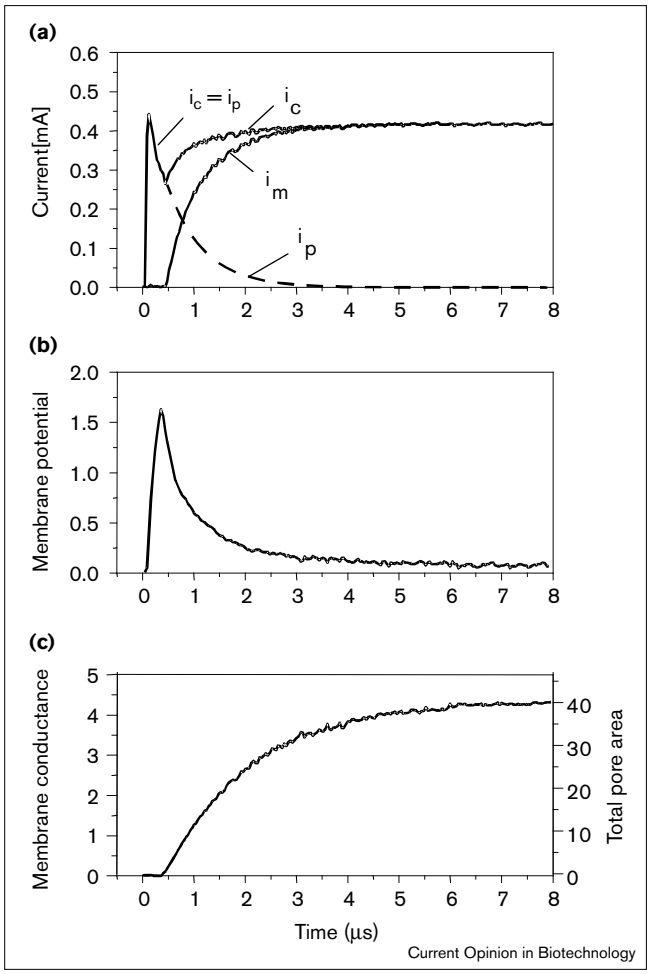
This method was used for the identification of the efficiency of HELP impact parameters (e.g. field strength, width, frequency or number of pulses) on biological cells [6••] and for the recording of rapid permeabilization changes during and after pulse application.

Data on cell permeabilization kinetics after HELP treatment of potato cell cultures are given in Figure 2. The build up of the membrane potential and pore formation in cells of potato tissue in the first microsecond after the initiation of pulsing with supercritical field strength is demonstrated in Figure 3 [7••]. Initially, the transmembrane potential, φ, increases exponentially with the time constant $\tau = 0.7$ μs, which is the charging time constant for intact membranes in potato cells. At near to 0.4 μs, φ reached 1.7 V as a and later decreased to a residual value of 0.1 V, as a consequence of pore formation and a drastic increase in membrane conductance.

Figure 4 provides a detailed insight into the build up and decrease of the membrane potential, the pore formation and the time-dependent resealing after the application of a single pulse.

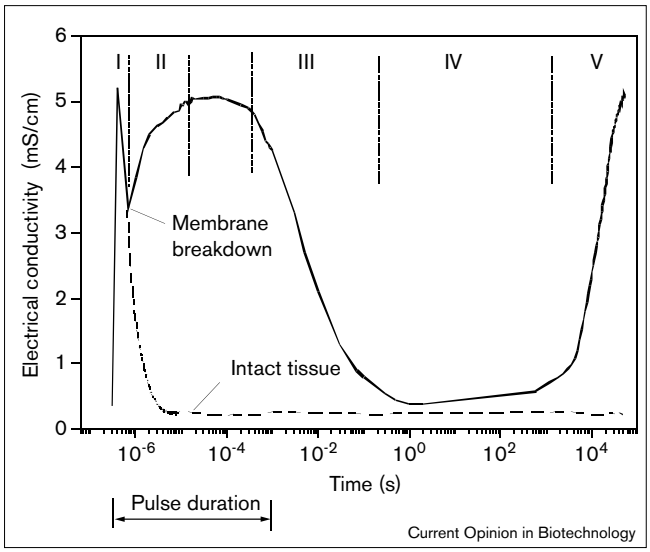
This is essential for process development as optimum repetitive pulse sequences for irreversible permeabilization can be developed based on these data. Furthermore, the

Figure 3



Current-voltage and membrane structure changes in an individual cell in potato tissue induced from external electrical field with field strength of 0.88 kV/cm [7••]. **(a)** Current and **(b)** transmembrane potential in the average cell. The membrane breakdown voltage is 1.7 V. **(c)** Membrane conductance and total pore area increase in the membrane surface in the field direction. i_c, intracellular current; i_m, membrane current; i_p, polarisation current.

Figure 4



Permeabilization characteristics in cells of potato tissue during and after pulse application. Peak field strength and duration of the exponential pulse were 0.88 kV/cm and 1 ms, respectively. The conductivity changes of intact tissue (broken line) is obtained by application of pulse at subcritical field strength of 0.1 kV/cm. I, membrane charging process; II, development of conductance membrane due the pore formation; III, resealing of the pore; IV, irreversible pore formation; V, stress induced indigenous (possibly enzymatic) membrane permeabilisation.

increase of the cell disintegration index within a few hours after HELP treatment resulting ultimately in irreversible permeabilization, offers the potential for unique and lower energy process and product development concepts (e.g. an increase in stress-induced metabolite production and concurrently improved extractability).

Enzyme inactivation in food products

Because pulsed electric fields affect the conformational state of proteins and enzymes, they can be used to prevent detrimental reactions that produce oxidation, off flavors, and color changes in food products [1•]. Various reports on the effects of HELP on enzymes provide mixed results. For example, Ho *et al.* [8] applied instant-charge-reversal pulse waveshapes and reported partial inactivation of lipase, glucose oxidase, α-amylase, peroxidase and phenol oxidase. In contrast, lysozyme and pepsin presented increased activities in certain ranges of applied voltage. Because the degree of denaturation varied from enzyme to enzyme it remains unclear whether inactivation levels are due to the structure of the proteins, the creation of active sites, the concentration of the treated enzymes, local heating effect because of treatment chamber designs or field distributions, or a combination of the above effects [1•].

Process and product concepts based on HELP

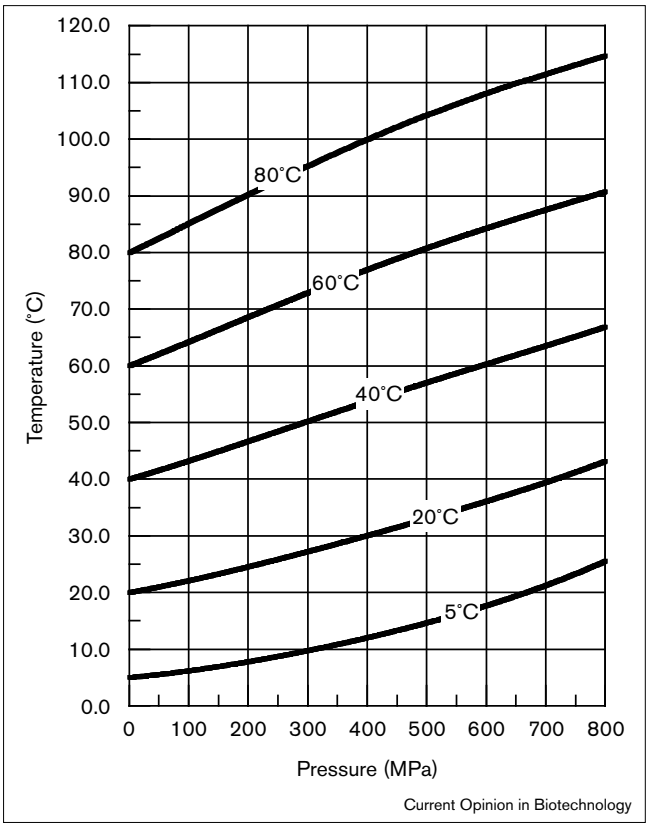
Because mass transfer can be influenced by HELP processes, diffusion or extraction rates as well as quantities of metabolites and drying of plant materials can be improved [2•]. We are also investigating the impact of reversible membrane permeabilization and the related potential for infusion operations of biopolymers into plant cells, as well as the stress response of the 'plant cell bioreactor' to subcritical field strength (i.e. increase of phenol concentrations).

High pressure processing

Scientists of the nineteenth century created a foundation for modern high-pressure physics and chemistry and began the study of the inhabitants of high pressure environments. Subsequently, animal and bacterial life has been identified from the Mariana Trench (at 110.6 MPa) and the Philippine Trench (at 101.7 MPa), respectively [9••].

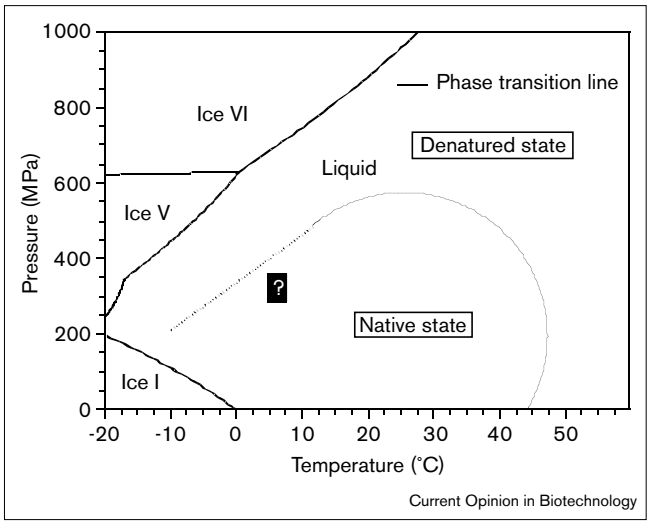
High hydrostatic pressure treatment of food systems was first reported 100 years ago by Hite [10] and has received

Figure 5



Adiabatic heating during compression of water at different initial temperatures.

Figure 6



Phase diagram of water and proteins under pressure.

considerable attention since it was rediscovered ~15 years ago. Since then it has been at the center of food research and development activities, especially since the emergence of commercial products (fruit preparations and juices) on the Japanese market in 1991. Initial emphasis was directed towards food preservation with the goal of extending product shelf life with minimum impact on product quality. Subsequently, the great potential of food and food constituents for physical modification of structure and function as well as the possibility for new process development (i.e. pressure-assisted freezing or thawing) has been recognized [11]. Meanwhile, commercial products (avocado puree, orange juice and milk) are also available in Europe and the USA. Batch and semi-continuous equipment is available on an industrial scale.

Pressure primarily affects the volume of a system. The influence of pressure on the reaction rate can be described by the transition-state theory, where the rate constant of a reaction in a liquid phase is proportional to the quasi equilibrium constant for the formation of active reactants. Based on this assumption, it was reported [12] that at constant temperature, the pressure dependence of the reaction velocity constant (k) is due to the activation volume of the reaction (ΔV*):

$$\left(\frac{\partial \mathrm{In}(k)}{\partial p}\right)_T = -\frac{\Delta V^*}{R \cdot T}$$

where p is the pressure, R is the gas constant (8.314 cm3/MPa/K/mol) and T is the temperature (K).

Table 1

Pressure dependency of water.

MPa	0°C			20°C			40°C			80°C		
	v (L/kg)	cp (kJ/kgK)	pH	v (L/kg)	cp (kJ/kgK)	pH	v (L/kg)	cp (kJ/kgK)	pH	v (L/kg)	cp (kJ/kgK)	pH
0.1	1.000	4.228	7.4	1.002	4.183	7.1	1.008	4.182	6.8	1.029	4.194	6.3
100	0.957	3.909	7.3	0.962	3.968	6.9	0.969	4.002	6.6	0.988	4.023	6.2
200	0.925	3.786	7.1	0.931	3.861	6.8	0.939	3.901	6.5	0.957	3.917	6.0
400	0.883	3.32	6.9	0.887	3.60	6.6	0.894	3.77	6.3	0.910	3.79	5.8
600	–	–	–	0.853	2.85	6.4	0.861	3.68	6.1	0.876	3.74	5.7
800	–	–	–	–	–	–	0.833	3.64	6.0	0.850	3.83	5.6

Pressure effects on water

The temperature-dependent compressibility of water is summarized in Table 1. Adiabatic compression of water increases the temperature ~3°C per 100 MPa (Figure 5). Self ionization of water is also promoted by HP lowering the pH. Phase transition of water can be performed under pressure. At ~1,000 MPa water freezes at room temperature, whereas the freezing point can be lowered to –22°C at 207.5 MPa. As evident from the phase diagram in Figure 6, this allows for pressure shift freezing of foods with instant and small ice crystal formation, storage of food at subzero temperatures without ice formation, or fast thawing of frozen food by pressurization. As discussed below, this occurs concurrently with HP-assisted control of microbial growth [13]. This allows gentle processing of foods or food constituents (i.e. starter cultures) with minimal structural damage.

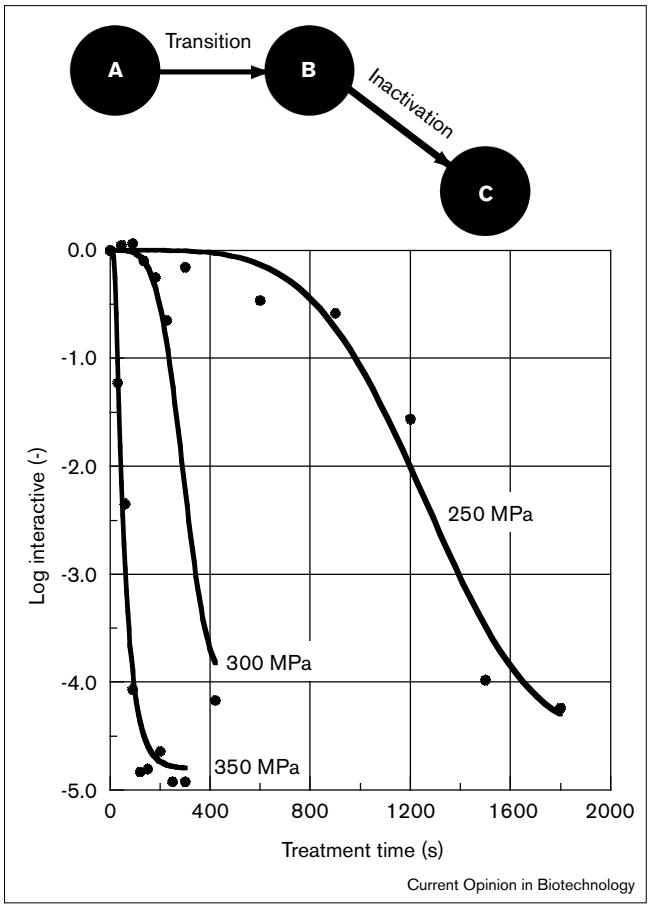
High pressure effects on microorganisms

Kinetic data on the inactivation of vegetative microorganisms have been accumulated in recent years and led to the development of a three-state inactivation model (see Figure 7; [14]). This model has been validated for several organisms in model systems as well as in real food systems. Data in Figure 8 also suggest that that microbial HP inactivation of certain pathogens, such as Listeria, can be favored at low (cold storage) temperatures. This phenomenon could be partly explained by the phase diagram of proteins (Figure 6); however, the HP inactivation mechanisms are more complex (besides protein denaturation), including morphological changes and membrane effects [11].

Smelt indicated increased lactic acid production of pressure treated *Lactobacillaceae* (JPM Smelt, personal communication). Because certain health benefits (e.g. reduced bacterial diarrhoea) could also be attributed to non-viable probiotic bacteria [15] the evaluation of metabolite production of HP-stressed microorganisms is of interest. In addition, Smelt also found practically no correlation between heat and pressure inactivation of 98 *Salmonellae* strains. This offers unique concepts for selective inactivation of certain microbial populations via pressure–temperature combinations.

Bacterial spores proved pressure resistant, but pressure-induced germination of spores has been reported in early HP studies [11]. Consequently, HP germination and inactivation kinetics have been recorded and a mechanism of HP-induced *Bacillus subtilis* spore germination has been proposed [16]. More recently, these data have been validated for other organisms (e.g. *Bacillus stearothermophilus* spores) and in real food systems, suggesting that a combination of heat treatment (90°C) with moderate pressures of 150 MPa can lead to direct inactivation of spores. Data obtained by X-ray microscopy (Figure 9) indicate that *B. subtilis* spores inactivated by a combination of 150 MPa and 70°C have lost the potential for germination.

Figure 7



Three state model of microbial inactivation (*B. subtilis*) under isobaric conditions [14]. A, stable; B, metastable; C, inactivated.

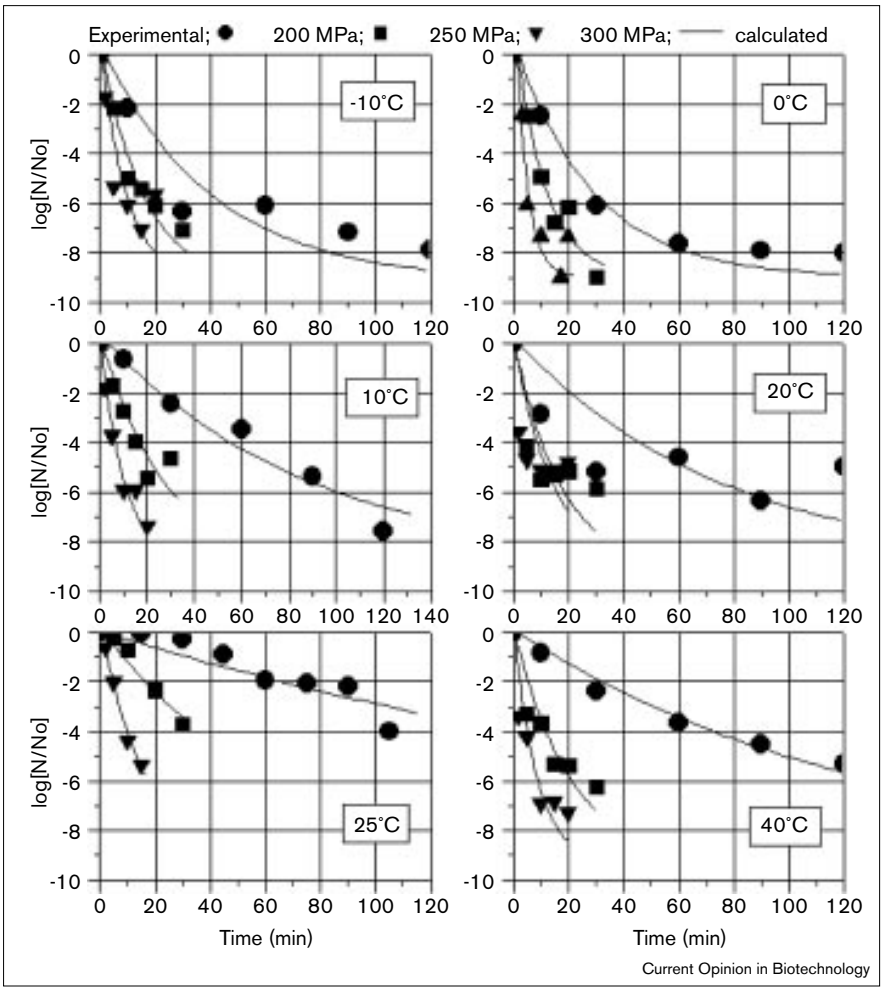
Interestingly, under these conditions dipicolinic acid — an indicator of germination — is being released. These observed effects provide interesting insights in the germination and inactivation of spores and require further study.

In Figure 9, the effect of a 5 min pressure treatment at 150 MPa on structural elements of the spore is presented (V Heinz *et al.*, personal communication). Compared to the control, the treatment at 30°C showed the onset of degradative events at the core, which is observable in X-ray microscopy by the reduced absorbance of the core of the spore. The viability is not affected unless the temperature in increased to 70°C. Treatment at 150 kPa and 70°C (Figure 9) killed three log-cycles of the population but the microscopic image gives no indication for the occurance of germination. It is assumed that vital parts of the enzymatic germination system [17] are affected by the combined action of pressure and elevated temperature, which prevents the spore from outgrowing [15].

High pressure effects on plants

Heat and mass transfer in plant tissues can also be influenced by HP, resulting in improved release of metabolites,

Figure 8



Pressure and temperature effects on the inactivation of *Listeria*.

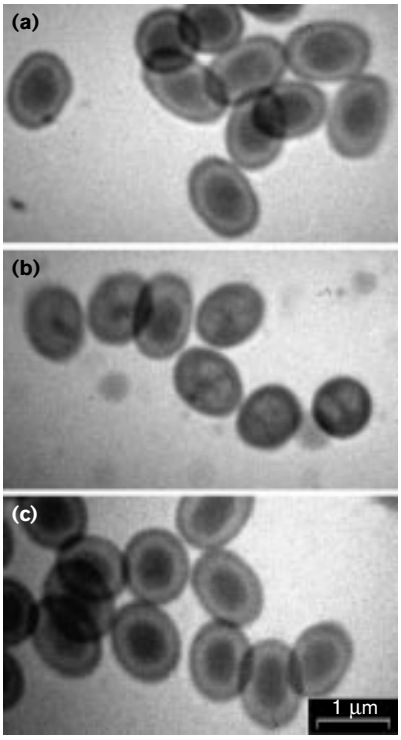
increased drying rates of vegetables, or reduced fat uptake of french fries. Furthermore, tissue hardening in vegetables and gel formation in fruit purees — partly due to pressure-induced release of calcium ions and residual pectin methyl esterase activity resulting in the formation of calcium-pectate — has been observeved [6••]. Using plant cell cultures as model systems, pressure effects on membranes as well as pressure-induced stress reactions are being evaluated in our laboratory. Special emphasis is on directed increase in the production of desired metabolites, such as phenols [18], by using pressures below the permeabilization pressure of the tonoplast at 100–150 MPa. Pressure induced gelation of starches [19], as well as of proteins [20] and protein–polysaccharide mixtures, offers a wide potential for modifying structure and function of biopolymers.

High pressure effects on proteins

Structural rearrangements taking place in proteins under pressure are governed by the Le Chatelier Principle. Accordingly, processes associated with a volume decrease are encouraged by pressure, whereas processes involving a volume increase are inhibited by pressure [20]. Covalent bonds are almost unaffected by high pressure and so the primary structure of proteins will remain intact during pressure treatment. Secondary structure changes occur at very high pressures, which might be explained by the rupture of hydrogen bonds (which are enhanced at low pressures). In general, pressures above 300 MPa cause irreversible protein denaturation at room temperature, whereas lower pressures result in reversible changes in protein structure. A lowering of the denaturation pressure with both decreases and increases in temperature (Figure 6) has been observed.

Effects of HP on enzymes have been divided in two classes. Relatively low pressures of 100–200 MPa have been shown to activate monomeric enzymes, whereas higher pressures generally induce enzyme inactivation. As for pressure-temperature combination effects, a pressure-temperature kinetic diagram of bacterial α-amylase has been exemplified by Hendrickx *et al.* [20]. As pressure resistance of enzymes is not related to thermal resistance, new processing and product options (i.e. biotransformation enhancements and structure engineering) as well as challenges (i.e. food quality changes) exist because of enzyme activation.

Figure 9



X-ray microscopic images of *Bacillus subtilis* spores. **(a)** Control. **(b)** After treatment: 30°C, 150 MPa, 5 min. **(c)** After treatment: 70°C, 150 MPa, 5 min.

Conclusions

Non-thermal processes, such as HELP and HP, offer a wide range of food and biotechnological applications, especially regarding membrane-related effects, sublethal stress-induced biosynthetic effects on plants and microorganisms, and a tremendous potential for physical or physico-biological modification as a result of temperature–pressure interactions (e.g. structure engineering). The pressure-assisted targeting of enzyme or microbial activities or the reversible permeabilization of biological membranes by HELP may allow intriguing uses. The application of the non-thermal processes has been suggested within the Scientific Concepts of Functional Foods in Europe [21••] to overcome the technological challenges of optimization of functional food components by increasing their concentrations in raw materials, through their modification and through increased bioavailability. Finally, the development of future technologies based on HELP or HP, such as pressure-assisted freezing, storing or thawing of sensible biological materials (i.e. biological tissues or organs), can be envisioned.

Acknowledgements

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- •• of outstanding interest

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