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High-pressure Adaptation of Extremophiles and Biotechnological Applications

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Abstract (about 150 words)

During the last decades, high pressure has been an important physical parameter not only to study biomolecules, but also for its biotechnological applications. High pressure affects organism's ability to survive by altering most of cell's macromolecules. These effects can be used, for example, to inactivate microorganisms, enhance enzymatic reactions or to modulate cell activities. Moreover, some organisms are capable to growth under high pressures thanks to their adaptation at all cellular levels. Such adaptation confers a wide range of potentially interesting macromolecules still to be discovered. In this chapter, we firstly present the different effects of pressure on cells and the diverse strategies used to cope against this harsh environment. Secondly, we explored the pressure biotechnological applications on pressure-sensitive and adapted-pressure organisms.

Keywords: high pressure, deep biosphere, stress, piezophile, adaptation

Introduction

High pressure (HP) characterizes many habitats on Earth, such as deep sea, sub sea floor and continental subsurface. Ocean covers approximately 70% of world's surface and its average pressure is 38 MPa (1 MPa = 10 bar). This hydrostatic pressure originates from the weight of the water column and corresponds to 10 MPa/km[1]. Part of the oceans are defined as "deep sea", which encompasses the entire biosphere below 1000 m from

the water surface and consequently with pressure higher than 10 MPa. The highest hydrostatic pressure detected in the ocean is approximately 110 MPa at 11,000 m depth at the Challenger Deep of Mariana Trench in the Pacific Ocean. Though, pressures above 110 MPa have been found at the sub sea floor. In this case, pressure is caused by hydrostatic pressure but also by the weight of material (lithostatic pressure) and it increases roughly by 15 MPa/km in sediments and by about 28 MPa/km in oceanic rocks. Sub sea floor contains a substantial part of the Earth biomass and can potentially influence global biochemistry [2–4].

All high-pressure habitats are occupied by microorganisms and other complex organisms and they highly contribute to the Earth's biomass [5,6]. Pressure impact on organisms' growth allow to divide them in different categories. Organisms that cannot tolerate ambient pressure are designed as strict or obligate piezophiles, inversely, facultative piezophiles or just piezophiles are organism that tolerates ambient pressure, but their optimal growth pressures are higher than 10 MPa (figure 1). On the other side, organism that withstand optimal pressures lower than 10 MPa are called piezotolerants and, finally, organisms which their growth is inhibited by pressure are designed as piezosensitive [7].

Figure 1. Schematic growth curves of microorganisms according to pressure (MPa) [7].

The interest in piezophiles has begun more than a century ago[8,9], but the technological difficulties and the need of specialized equipment have done that high-pressure studies are not, currently, developed in most laboratories. However, the interest and the applications of the pressure biotechnological applications have been growing during last decades.

Bacteria and Archaea domains contain facultative and obligate piezophiles. Examples of obligate piezophiles are the bacteria *Shewanella benthica* and *Colwellia marinimaniae*, with their optimal pressure at 70 MPa and 120 MPa respectively [10,11], and the archaeon *Pyrococcus yayanossi*, withstanding an optimal pressure of 50 MPa [12]. The technical constraints to isolate obligate piezophiles may provoke their underestimation. Obviously, piezophiles excel in sustaining pressure conditions beyond the usual limits for humans; however, the reasons for that adaptation are still debated.

1. Effects of Pressure on Macromolecules and Cells

Pressure alters the biomolecules by changing their volume. Thermodynamically, the variation in Gibbs free energy (G) is defined by the equation 1

$$d \Delta G = -\Delta S dT + \Delta V dP, \quad (1)$$

where ΔS is the difference in entropy, ΔV the change in volume and T and P represent temperature and pressure, respectively. At constant temperature, $dT = 0$ and thus

$$\left(\frac{\partial G}{\partial P}\right)_T = \Delta V. \quad (2)$$

This equation, according to the Le Châtelier principle [13], states that an increase in pressure will cause a shift to the state that occupies the smallest volume, meaning, for example, to the unfolded state for most globular proteins, where $\Delta G < 0$. As a result, pressure modifies the volume of the system but not its internal energy (as temperature does). Water with its low compressibility is a crucial partner for pressure action. Notably hydration water (water bound at the surface of macromolecules) is very sensitive to pressure and it can reorganize its network under pressure implying an effect on the macromolecule[14]. Moreover, macromolecules present an extraordinary stability against pressure under low hydration conditions [15,16].

Relatively low pressures affect the interatomic molecular bindings (such as Van der Waals, hydrogen bonding and hydrophobic interactions) altering conformations and structures of biomolecules and therefore, their physical properties (solubility, melting point, density), equilibrium states and processes' rate [17]. Electrostatic and hydrophobic interactions are specifically affected by pressure. In contrast, pressures above 2 GPa are needed to impact non-covalent interactions [18,19].

1.1. Nucleic acids

Although the unfolding volume of DNA duplexes is small, pressures up to 1 GPa have, in general, a stabilizing effect on canonical DNA (e.g. with common pair bases). This stabilizing effect may be explained by the decrease of hydrogen bonds' distance.

Consequently, pressure increases the duplex-single-strand transition temperature[20]. Only in specific cases of synthetic polymers (e.g adenine – thiamine copolymer) and salt concentrations, pressure can lead to double-stranded melting [21]. Regardless, the mechanism is not the same as the heat-induced DNA melting, as under pressure, water molecules penetrate DNA base pairs destabilizing their interactions [22,23].

There is a lack of information about the effect of pressure on RNA but, generally, it has been observed that RNA is more pressure sensitive than DNA. For example, pressure induces a structure reorganization of tRNA [24,25] and it destabilizes small RNA oligomers [26].

Non-canonical pair structures (different from the usual Watson-Crick pair bases), such as G-quadruplexes or stem-loops, are less stable under pressure than canonical structures by a factor of 10 [22,27].

Although canonical DNA duplexes are stabilized by pressure, DNA—protein interactions may be perturbed due to changes in the electrostatic and hydrophobic interactions. Accordingly, pressure affects negatively all molecular reactions where DNA is involved, such as replication, transcription and recombination [28,29].

1.2. Proteins

Most of the knowledge about pressure effects on proteins is based on studies on globular proteins[30–32]. Some of these studies reveal that structural transitions of globular protein due to pressure are based on a hydration mechanism that accompanies protein conformational changes. At higher pressure the hydration degree is increased by the penetration of water into the protein cavities causing the increase of the surface area in contact with the solvents thus contributing to the volume change[33]. Pressure mainly alters tertiary and quaternary structures of proteins but secondary structures (α -helices,

β-sheets, and turns) are much less sensitive to water penetration and to destabilization by pressure. For this reason, the state unfolded by pressure may be a hydrated globular structure with large amounts of folded structure[17,34]. It is important to note again the role of water on pressure denaturation, since this volume change can only be observed for proteins in solution. Dry proteins are highly stable against pressure[18,35,36].

The unfolding of many monomeric proteins begins above 200 MPa [4], however enzymatic activities are usually modified at lower pressures. In fact, the application of pressures < 200 MPa confers higher thermostability to most proteins [35,37]. Consequently, superposing pressure and temperature usually accelerates most of enzymatic reactions, such as hydrolases and transferases reactions [36]. Moreover, few reactions can be enhanced by pressure even at low temperatures [36]. For example, the efficiency of coconut husk hydrolysis by cellulases from *Penicillium variable* is increased at 300 MPa [38].

Pressure generally changes the equilibrium between oligomers and their subunits or between two different proteins and this even at relative low pressures (about 50 MPa) [39]. At this pressure, for example, ribosomes' subunits are dissociated [29] and larger protein assemblies as cytoskeletal proteins are disturbed resulting in reversible morphological changes [40]. However, other oligomers are more resistant to pressures as the tetrameric urate oxidase, which dissociates at about 150-175 MPa [41].

Actually, a protein in its native state possesses distinct, nearly isoenergetic conformational substates, which may have similar or dissimilar functions or the same function with different rates (statistical substates). As pressure can decrease the folding rate and increase the unfolding one, it can shift the population of different protein substates on the basis of their volumetric differences [42–44]. This capability allows the characterization of various intermediate substates by pressure, which may act in the

folding process [45]. Moreover, pressure can change the reaction rates, providing new information about the dynamics and reactions of proteins [34]. This was confirmed, for example, by a dynamic study of myoglobin, where it has been shown that pressure reduces protein motions and facilitates access to different substates [44].

Few studies have been done on pressure effects of non-globular proteins, as fibrous, disordered and membrane proteins. Examples are the studies on the collagen structure [46], the intrinsically disordered protein alpha synuclein [47] and on the Lmr transmembrane protein [48] or the ion channel MscS [49]. An important point is that the behaviour of transmembrane proteins against environmental stresses is affected by the protein structure but also by its lipids surrounding [50].

Membrane proteins and membrane lipids form an ensemble; they influence each other as a result of biochemical or environmental changes which can compromise membrane process as energy production or ion flux. For example, the transporter efficiency of the tryptophan permease Tat2 from yeast cells is affected due to a membrane fluidity modification at HP[51]. Therefore, membrane integrative studies are necessary to better describe the membrane protein behaviour. Its influence of the lipid matrix on the protein response to pressure have been studied for few proteins [52–54].

1.3. Phospholipids

Lipids, and specially their hydrocarbon chains, possess a highly compressible potential, which makes them very sensitive to pressure [55]. When pressure is applied on a phospholipid bilayer, the acyl chains from phospholipids get straighten resulting in a thicker and higher ordered bilayer. Due to this higher acyl chains' order, pressure promotes the lipid phase transition from liquid-crystalline (phase essential for the biological function of the membrane) to a more rigid phase called the gel phase.

172 Additionally, pressure can also promote the apparition of new phases, such as
173 interdigitated phases or non-lamellar phases (i.e., cubic or hexagonal)[55–59].
174 Nevertheless, not all lipids have the same sensitivity to pressure, for example, lipids
175 with longer hydrophobic chains are more responsive to pressure. This may result in a
176 phase separation in domains on model membranes from a mixture of lipids[60].

177 Pressure may have also an impact on more complex macromolecules, as lipoproteins.
178 Recent studies on human plasma lipoproteins under HHP revealed a reduced flexibility
179 and higher compressibility of its triglyceride rich form, the form associated to
180 pathological health conditions [61,62].

181 **1.4. Cells**

182 Surprisingly, pressure is the unique physical parameter capable of inducing heat-shock
183 and cold-shock proteins' as a cell response to a same stress. *Escherichia coli* (*E. coli*)
184 exposed to 53 MPa induces 55 proteins, 11 heat-shock and 4 cold-shock proteins among
185 them. *E. coli* may try to counteract the damage produced by pressure at different cell
186 levels, such as stability of macromolecules and membrane functionality[63].

187 As mentioned, pressures up to 100 MPa affects most of the cellular functions as
188 enzymatic reactions, gene expression, cell motility and morphology, and cell membrane
189 (figure 2). Since pressure is transmitted through a fluid, it will be transmitted uniformly
190 (Pascal's law) over the whole cell and therefore, it makes difficult to identify, if it
191 exists, the main cause of the cell death. Moreover, pressure-induced cell inactivation
192 relies on the type of microorganism and its physiological conditions, such as water
193 content and salt presence.

194 Overall, eukayotes are more pressure-sensitive than prokaryotes and piezosensitive
195 bacilli and spiral-shaped bacteria are inactivated at lower pressures than cocci [39,64].

196 For instance, pressures above 150 MPa usually reduces the viability of mammalian cells
197 and may induce cell death by apoptosis from 200 MPa or through a necrotic-like
198 pathway at 300 MPa [65]. On the other hand, bacteria cocci may resist much higher
199 pressure variations, for example, *Staphylococcus aureus* cell inactivation begins at 350
200 MPa [64].

Figure 2. General effects of pressure on cellular macromolecules (black) and cells (red). Adapted from [66]

In addition, most gram-negative bacteria seem to be less resistant to pressure than gram-positive [67]. Gram-negative bacteria possess a much more complex membrane which makes it a target for pressure damage [68]. Finally, microorganisms on the exponential

growth phase present lower pressure-tolerance than on their stationary growth phase[69,70], for example, exponential-cells may present filamentous shape under pressure which can disrupt membrane functions [29,71]. Moreover, stationary-cells have the capability to synthesize stress-response proteins to adapt and, therefore, better resist to different harsh conditions[72].

Spores present formidable high resistivity to harsh environments, likely due to their structure with numerous protective layers and their low water content [73]. Interestingly, relative moderate pressures (50 to 300 MPa) cause the germination of a dormant spore. Though, higher pressures are often less effective to induce germination[74]. Pressure alone is not very effective to inactivate bacterial spores and a treatment with temperature is necessary[75].

2. Pressure Adaptation in Piezophiles

The biodiversity of piezophiles is huge [5,76–78]. Organisms adapted to pressure include unicellular bacteria, archaea, eukaryotes as invertebrates and fishes and even deep diving marine mammals [79–81]. For example, in several hydrothermal vents have been found large invertebrates like mussels, crabs and shrimps and some marine mammals can dive exposed to almost 20 MPa without any negative symptom [82,83]. There are differences in the microorganisms adapted to HP, as some are also adapted to low temperatures (psychrophiles) and others to high temperatures (hyperthermophiles), which increases the piezophilic diversity. Hyperthermophilic and piezophilic organisms are found near vent sites, where temperatures can go from 350 °C to 2°C in only few cm distances. Altogether, this represents a source of microorganisms with great potential

for technological and pharmaceutical applications, such as new enzymes, antibiotics or cancer cell line active derivatives.

Figure 3. Optimal growth temperature and pressure for some piezophilic bacteria (blue) and archaea (red).

Most piezophiles identified in the deep sea are bacteria, they are psychrophiles and piezophiles, but in the niche of hydrothermal vents, most microorganisms are archaea, they are thermophiles and piezophiles [84] (figure 3). This indicates that piezophiles may follow a different adaptation to temperature and makes it difficult to separate the adaptation to pressure from other stress adaptation since microorganisms may adopted a common strategy to cope with various environmental stresses [85]. High pressure has a close relation with other stresses as temperature, pH and salt. Moreover, there are relatively few studies on adaptation mechanisms to pressure and not all adaptations are equally used by the different piezophiles studied. Additionally, not all necessary pressure adaptation mechanisms are deleted at ambient pressure, and therefore the homologue enzymes may use the same mechanism [66]. Finally, piezophilic biospheres

are closely related to nutrient limitation and most piezophiles must also be oligotrophes [4].

Extremophiles have developed great capabilities to adapt to harsh and even fluctuating conditions (e.g. temperature, pressure, composition of the host rocks...) thanks to their own unique macromolecules, such as polysaccharides, lipids or enzymes and even specialized organs[86]. These macromolecules adapted to HP present a high potential to develop new biotechnological applications.

2.1. Genomes

Marine biosphere contains mobile genetic elements, such as plasmids, bacteriophages, transposons, integrons, integrative conjugative elements and genomic islands [87]. All of them are essential to understand the high marine microbial diversification and thereby their adaptation [88,89]. Consequently, horizontal gene transfer of these mobile genetic elements may play an essential role in the microbial adaptation to pressure and other extreme conditions [90].

Recently, it has been described that the gene Ypr153w is possibly responsible for the tryptophan permease's Tat2 stability in *Saccharomyces cerevisiae* under pressure. It is a gene which has also been identified in other related species as *Debaryomyces* and *Candida* strains which have been isolated from sediment samples of deep sea floors [91]. Another possible HP adaptation could be the 16s rRNA longer stems found in strains from *Photobacterium*, *Colwellia* and *Shewanella* [92].

The high genetic tractability and hyper-responsiveness to pressure of the piezophile *Photobacterium profundum* strain SS9 has made it a reference for studies on piezophile adaptation. It has been discovered a pressure-sensitive mutant of SS9 that lacks the recD gene, responsible for a DNA-binding protein. Besides, the transfer of the recD gene

from SS9 to *E.coli* enabled the latter one to divide normally under HP. This indicates that RecD may have an important role for piezoadaptation together with a role of DNA metabolism and cell division[93,94].

To date, it has not been possible to detect any piezospecific gene. Consequently, it is not possible to determine if an organism is piezophile by molecular approaches, it is necessary to do cultivation approaches and to determine the growth rates at different pressures. However, it has been found that the pressure regulated operons ORFs 1–3 are distributed among different piezophilic *Shewanella* species[95].

2.2. Proteins

Relatively few enzymes from piezophiles have been studied under pressure. Although there are no apparent differences between the crystal structure of an enzyme from a piezophile and its piezosensitive homologue, there is a variation in the stability between both enzymes caused by a difference in flexibility and hydration of the proteins[96]. Most molecular motion studies about pressure adaptation have been done *in vitro*, or investigating, for example, molecular dynamics[97]. Nevertheless, nowadays *in vivo* studies have gained importance thanks to, for example, neutron scattering and NMR experiments that can examine timescales from few nanoseconds to hundreds of milliseconds[98–100].

It has been shown that some proteins are involved in HP adaptation as well as in adaptation to other stresses (Hsp60, Hsp70, OmpH, RecA, F1F0 ATPases, Cct and Tat2)[101]. A system highly studied under pressure is the Omp/Tox system. The proteins ToxS and ToxR from *P. profundum* SS9 are responsible for regulating the genes that encode the membrane proteins OmpH, OmpL and OmpI. Pressure reduces the abundance and the activity of ToxR, which therefore upregulates the protein OmpH

among others, the system acts as a piezometer. Regardless, the systems ToxS and ToxR do not confer HP adaptation and their role under pressure is not clear [8,102].

The protein adaptation to extreme conditions is a balance between the imperative stability (higher number of bounds) to be functional and the flexibility (lower number of bounds) to be capable to adapt to different conditions [103]. One of the most studied enzymes is dihydrofolate reductase (DHFR). Studies comparing DHFR from the piezosensitive *E. coli* and from the facultative psychro-piezophile bacteria *Moritella profunda* reveals that applying pressure decreases EcDHFR activity and increases MpDHFR activity up to 50 MPa before diminishing its activity at higher HP. MpDHFR seems to have higher sensitivity to pressure due to its higher flexibility [104]. A more flexible protein may explain the higher absolute activity of piezophile proteins [105].

However, most of studies are done in protein-isolated solutions, which differs from their native state. An innovative quasi-elastic neutron scattering study examined the dynamics from whole cells of the piezophile *Thermococcus barophilus* and the piezosensitive *Thermococcus kodakarensis* microorganism under atmospheric pressure and 40 MPa. This study revealed that the HP adaptation on whole cells is based on an overall higher proteins' flexibility and, in addition, on the modification of their hydration layers [99].

Proteins from piezophiles may have a larger total volume of small internal cavities, which makes the protein more compressible and less sensitive to distortion caused by pressure [105]. Moreover, the presence of small cavities allows water penetration at HP and consequently increases the hydration but, as seen in MpDHFR, cavities are not big enough to cause the protein denaturation but allow the protein to be more flexible. The presence of more small cavities could decrease the amount of water molecules contained in each cavity (a volume of $15\text{\AA}^3$ is necessary for a single water molecule and

an increase of approximately $45\text{\AA}^3$ is required for each extra molecule [106]). It is important to consider that cavities are not mere “packing defects” but that they play a role in conformational changes and in controlling binding and catalysis of the proteins [106,107].

Generally, monomeric proteins are more resistant to pressure than oligomeric proteins. However, it has been shown that multimeric proteins may be adapted to resist to pressure. For example, studies on the hyperthermophile and piezophile TET3 peptidase from *Pyrococcus horikoshii* indicate that the protein multimerizes into a dodecamer structure instead of conserving its classical barrel-shape multimer conformation. Dodecamer multimerization protects the hydrogen bonding between the different subunits and increases its stability against temperature and pressure up to 300 MPa [108].

A general extrinsic cell response to pressure-stress is the presence of piezolytes and other low weight organic compounds called osmolytes (e.g., sugars and amino acids) to protect the cell macromolecules, such as proteins, from pressure modification [109] and therefore adapt its dynamics. Some piezophiles accumulate these low-weight molecules in response to an increase on pressure and others to a decrease, indicating in the latter case that the growth at lower pressure than optimal is perceived as a stress for these piezophiles. For example, trimethylamine oxide (TMAO) is a pressure co-solute that helps proteins to remain active under HP in certain fishes and crustaceans [80,110]. On the other hand, the hyperthermophilic and piezophilic *Thermococcus barophilus* accumulates mannosyl-glycerate when it is grown in non-optimal conditions (ambient pressure) [111]. This indicates that this archaeon perceives the lack of pressure as a stressful condition.

Few studies have been done on pressure adaptation of higher complex pluricellular organisms. For example, it is thought that the regulation of N-methyl-D-aspartate receptor (NMDR), a cell membrane protein found in nerve cells, is responsible for the absence of the high-pressure nervous syndrome (HPNS) on deep dive mammals [83,112]. The regulation of this protein may be done by modulating its interaction with lipids, for example by the presence of cholesterol, and thanks to protein's particular tertiary structure in piezo-tolerant organisms.

2.3. Membrane lipids

Cells have the capability to modify their cell membrane lipid composition metabolically to maintain it in a functional liquid crystalline phase with specific functional physicochemical properties, such as fluidity, permeability and membrane curvature in spite of environmental stresses. This process is known as homeoviscous adaptation [113].

Eukarya and bacteria possess lipids different from those in archaea but their homeoviscous adaptations have similarities. Eukaryal and bacterial lipids are composed by straight hydrocarbon chains linked by ester bonds on 1,2-*sn*-glycerol and a phosphodiester-linked polar group or sugar. On the other hand, archaeal lipids have isoprenoid hydrocarbon chains bounded by ether bonds on 2,3-*sn*-glycerol. Partly, the adaptation of archaea to extreme conditions may thus rise from their particular lipid structure [114].

The common routes of lipid adaptation of bacterial and archaeal membranes are the change of the acyl chain length, the addition or removal of mono-unsaturated lipids and the change in the polar headgroups [115,116]. Longer acyl chains are translated into more rigid membranes, in contrary adding just one unsaturation to lipid chains makes

the membrane more permeable and larger headgroups increase the disruption of the membrane packing by increasing the membrane fluidity. Furthermore, archaea possess tetraether lipids, which may form a monolayer instead of the common bilayer. In addition, some archaeal species comprise lipids with cyclopentane rings and isoprenoid chains that are crosslinked. The change in the different ratios from di- and tetra-ether lipids and the presence of cyclopentanes and crosslinked chains modifies as well the properties of the cell membrane. Finally, psychrophilic bacteria present polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which, just as lipids with one unsaturation, increase the permeability of the membrane under low temperatures[117]. The function of PUFAs is not clear, one of the hypotheses is that requires less carbon and energy to obtain the same effect than the mono-unsaturated lipids [8,117,118]. Another hypothesis is that they may play a role in cell division under HP, as demonstrated for bacteria *Shewanella violacea*[119].

Only two studies have been done to examine the lipid composition under pressure: one on *Methanocaldococcus jannaschii* and another on *T. barophilus*[120,121]. Both present an increase in the diether : tetraether lipid ratio to counteract the increase in rigidity provoked by pressure on the cell membrane.

3. Pressure Biotechnological Applications

HP application is mostly used in food processing since it does not affect non-covalent interactions (at least up to 2 GPa) and it can inactivate bacteria and viruses without changing markedly nutrients and flavours of food. Furthermore, pressure can change the reaction rates, which may favour the extraction of the required product[19].

Besides, pressure may be used for diverse biotechnological and biopharmaceutical applications, for example to explore new therapies [122,123] and conserve vaccines [123], improve cryopreservation [125] or for orthopaedics' surgery [126].

3.1. Food Industry

HP (400 — 600 MPa) inactivates microorganisms, like yeast, molds and viruses. It affects the cell at different levels, such as nutrient transport and cell reproduction, reaching to the cell death [75,127]. Moreover, HP hardly affects low-molecular weight compounds (ex: vitamins, amino acids, flavour molecules) so, organoleptic and nutritional properties are only slightly modified [128]. On the other hand, HP alone cannot inactivate bacterial spores and thus a combination with other variations of pH, chemicals or thermal processes maybe needed. Nevertheless, pressure reduces considerably the working temperature, as 70 °C instead of 180 °C is enough to inactivate spores if it is combined with 600 MPa [75]. Such decrease in temperature can help to preserve quality and minimise off-flavour generations. Therefore, HP techniques are useful as a complement on thermal process but also to inactivate microorganisms on products where temperature cannot be applied. As an illustration, high pressure pasteurization of cold-pressed juices eliminate pathogens of juices without impairment of its fresh-like qualities and increasing the shelf life of the product [129,130].

HP extends shelf life of a high variety of food products. For example, fresh shrimp treated at 435 MPa has a shelf life of 15 days, three times longer than the shelf life of the untreated shrimp[131]. Fresh cheese treated at 300–400 MPa has a shelf life at 4 °C of 14–21 days, which is greatly higher than the 7 days for the untreated cheese[132].

Food is a complex matrix and inactivation efficiency depends on different factors as treatment conditions, microorganisms to inactivate and its food matrix characteristics. For example, meat treated at 300 MPa has a cooked like appearance but if it is processed at 100–200 MPa and 60 °C, it is more tender than the untreated meat[133]. Consequently, inactivation conditions must be defined for every food product. HP is not efficient for low water content food (such as flour) or food with high content of air bubbles and if the food needs to be wrapped before treatment only plastic packaging is acceptable, as packaging material needs a compressibility of at least 15% [127].

Finally, HP may also be applied as a pre-treatment. On the one hand, it has been demonstrated that the application of sublethal HP on cells gives them cross-resistance to other stresses. For example, the most studied probiotic, *Lactobacillus rhamnosus*, is more resistant to heat after an application of 100 MPa for 10 min[134]. This opens the possibility to inactivate the pathogens on probiotic products, conferring a health benefit.

On the other hand, pre-treatment can be useful to facilitate the extraction of internal nutritional components. HP makes the cells more permeable, increasing the mass transfer rate and as a result increasing the release of extracts. It has been shown that the time extraction of caffeine from green tea leaves is reduced from 20h to 1 min if a pressure of 500 MPa is applied; extraction of anthocyanin red grape skin is increased by 23% by applying 600 MPa of pressure and the extraction yield of ginsenosides from *Panax quinquefolium* root increases linearly between 100 MPa and 500 MPa [135].

3.2. Allergenicity and digestibility

Several proteins can provoke allergic reactions caused by an immune disorder on the IgE binding. Because HP tend to denature proteins and as a result, it has been shown to induce a modification of their allergenicity[136], both on protein solutions and on food systems. For example, pressures of 300–700 MPa reduce the allergenicity of a ginkgo seed protein and of soybean allergens[137]. Another interesting example is the use of HP together with proteases to obtain hypoallergenic rice[138,139]. However, the effect of HP on allergenicity is not universal. There is no allergenicity change caused by pressure on almonds, or on the protein Mald1 from apples[140,141]. Mald1 native state possess a high internal cavity occupied by water [142] and therefore, pressure may not be able to conform significant hydration changes since the protein is already highly hydrated.

HP may have not only an effect on the allergenicity of the food products but can help to increase its digestibility by exposing inaccessible sites of proteins and, thereby, enhancing the efficiency of protein hydrolysis. For example, the time required for proteolysis of β -lactoglobulin, the major allergen in cow's milk, is reduced from 48h to 20 min at 200 MPa [143].

3.3. Antiviral vaccines

Several viruses are inactivated or dissociated by pressure. Under pressure, the atomic contacts between subunits are replaced by interactions with the solvent and therefore once pressure is released, viruses cannot come back to their native form. For example, pressure inactivates picornaviruses by causing the lack of VP4 from the intern capsid [144]. Both viruses with polyhedral and helicoidal symmetry are sensitive to pressure.

Even so, not all viruses are equally reactive to pressure, for example, the foot-and-mouth-disease picornavirus is highly sensitive and poliovirus is much higher resistant against HP[144].

Interestingly, virus re-associate under their fusogenic state under pressure, a less infectious and highly immunogenic form[144–146]. This is why high pressure has been suggested for antiviral vaccine development. It has been demonstrated that immunization against HP-inactivated virus is equally effective as against intact virus and have higher immunity response than isolated viral subunits [42,147].

3.4. Bio-purification

An antigen may be purified from its medium by affinity chromatography due to a steric recognition with an antibody linked to a matrix. The recognition causes an increase in molecular volume and, as pressure causes a volume decrease, it could be useful to apply pressures to dissociate the product of interest without using drastic elution process which reduce the lifetime of matrices [148]. This has been demonstrated for the recovery of β -galactosidase: four 15 min cycles of 150 MPa at 4°C recovers 32% of *E. coli* β -galactosidase compared with the 46% recovered by adding a solution of pH=11 [149]. Although the product yield is lower when using HP, the method is simpler and have a lower impact on matrices than the current elution process.

HP ability to disrupt immune complexes has been proved on anti-prostate specific antibody from its antigen [149,150], its dissociation was increased by 22-37% when pressures from 140 to 550 MPa were applied. Pressure may also optimize the dissociation of amphiphilic biomolecules from a fixed adsorbent: 80% of Triton-X can be recovered from a bed absorption if a pressure of 250 MPa is applied on the system [151].

Finally, as pressure can dissociate aggregates, it may be used for the recovery of proteins from inclusion bodies, i.e. aggregates of incomplete folded proteins. Traditionally, to separate proteins from inclusion bodies is necessary to use high concentrations of agents that destroy the spatial structure of proteins with a necessary subsequent difficult refolding. However, a pressure of 240 MPa is effective to dissociate the inclusion bodies of endostatins and a subsequent application of 40 MPa induces the refolding of 78% of the protein [152].

3.5. Modulation of Cell Activity

Already relative low applied pressures can enhance the cell activity to our profit, as for example observed at 10 MPa for ethanol production by *Saccharomyces cerevisiae* [153] which occurs 3 times faster than at atmospheric pressure. Another example is on the fermentation by *Clostridium thermocellum* [154], this *Clostridium* converts cellobiose to biofuels and other chemicals but also synthesizes other non-desired products (acetate, H₂, CO₂). When the fermentation happens under pressure of 7 or 17 MPa, the microorganism modifies the metabolic pathways and shifts the production to desired metabolites, reaching an increase of 60-fold.

However, as HP is considered a stress for most cells, it will translate into the expense of additional energy for cell maintenance and growth, reducing the product yield. For example, HP reduces the fermentation rate of lactic acid fermentation due to the inhibitory effect on the growth of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium lactis* [155]. To avoid the loss of efficiency rates under HP, efforts are made to enhance the resistance to HP of mesophilic microorganisms, leading to HP resistant organisms with higher performance under HP [156].

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4. Biotechnological Applications of Piezophiles

There are more than 3000 enzymes identified to date and most of them are used for biotechnological applications. Nevertheless, these enzymes are not enough to respond to the new technological challenges that appear each day [157]. One of the problems is the stability of the enzymes under industrial conditions, so it is necessary to find enzymes which are highly resistant to harsh conditions and here deep-sea enzymes may play a major role. Pressure-stable enzymes are capable of sustaining biocatalysis under HP, modifying therefore specific enzymatic reactions, and have even higher thermostability. For example, Biolabs® has already commercialized a DNA polymerase from a hyperthermophile and piezophile *Pyrococcus*, which presents a half-life of 23 hours at 95 °C. Moreover, piezophilic enzymes may possess different properties than their surface homologues, which may open new possibilities for industry[158]. The market for industrial enzymes is growing every year and the exploitation of extremozymes is a huge and mostly unexplored resource[159].

As we have seen, lipids from extremophiles are unique. Archaea in particular contain lipids which confer to the cell a highly stable and impermeable membrane. The unique stability may be used in biotechnological or pharmaceutical applications, for example to protect therapeutic peptides from the harsh environment of the gastrointestinal tract[160,161]. Additionally, many piezophilic bacteria contain omega 3-PUFAs associated with their cell membrane, which are precursors to hormones and hormone-like molecules in many animals. Consequently, it could be used for hypertriglyceridemia diseases and clinical studies for this purpose have already been approved[158].

The high marine biodiversity has woken up the interest to search new compounds with biopharmaceutical potential[162]. It has been discovered marine derived molecules

with, for example, antitumor potential, for the treatment of pain or antimicrobial activities [158,161,162]. For example, studies have identified some bioactive compounds from marine echinoderms (such as the piezotolerant *Cucumaria frondosa*) with antiproliferative, antimetastatic and immunomodulatory activities[163].

All pressure-specific impacts allow to modify macromolecules and cells in unique ways. Food industry was pioneer in using pressure to inactivate microorganisms and as a pretreatment, but pressure capability does not stop here. Promising applications, such as antiviral vaccines, the use of pressure for bio-purification or to vary cell activities has led to a greater interest on this physical parameter. In addition, piezophile organisms opens a range of possibilities to use pressure-adapted molecules, and to increase, for example, the reaction rates, or to find new active macromolecules.

EXTRA

Biopharmaceutical

Bacterial ghosts as delivery systems

Bacterial ghosts are usually obtained by the expression of a lyses gene that leads to the formation of a transmembrane tunnel with its consequent cell material leakage. These products retain their immunogenic properties as their cell surface is not affected. Bacterial ghosts obtained by the expression of lysis gene does not retain their cellular structure and are permeabilized and therefore lose their immunogenic properties. HP is capable to prepare bacterial ghosts without the disadvantages of lysis gene, which make them a good option to use as delivery systems for subunit or DNA vaccines. For example, HP bacterial ghosts have been obtained applying a pressure of 100 MPa for 15 min to E.coli.

Cryopreservation

Oocyte cryopreservation by vitrification is one technique used to maintain women's fertility but blastocyst formation rate after this process is still low. This low formation rate is caused by the production of ROS components. As remarked above, sublethal HP stress makes the cells more resistant to thermal treatments and oocytes are not an exception. For example, pig oocytes, mouse and bovine blastocysts show a higher resilience against cryopreservation after being subjected to sublethal hp treatment. Moreover, It has been demonstrated that HP (20-40 MPa for 90-120 min) treated bull and boar spermatozoa before cryopreservation preserve the viability, motility and fertility after thawing.

Vaccines preservation

HP can be also considered as a stabilizing method for vaccine preservations. It has been demonstrated that high pressure stabilizes attenuated poliovirus against temperatures of 37°C, giving a higher thermal resistance to this virus. Actual trivalent oral polio vaccine (OPV), as most live vaccines, is heat labile, particularly OPV is between the most heat labile vaccines and it needs to be stored frozen and used

immediately after thawing. HP treatment may solve the problems of refrigeration guarantying a higher stabilised and effective poliovirus vaccine.

Oncology

Dendritic cell based vaccines

Shinitzky and colleagues have been explored the use of HP killed tumor cells as a whole cell vaccine, they have demonstrated that a pressure of 100-200 MPa increases the cell immunogenicity. However, since their phase I study in 2000, there are no more clinical studies using this approach.

Recently, dendritic cell (DC, antigen presenting cells from the mammalian immune system) based vaccines have been studied to obtain a vaccine for tumours. This approach uses pressures of 200 – 300 MPa to kill cancer cell lines, pressure will reveal immunogenic sites on the killed cancer cell surface. Then, these molecules interacts with the specific receptors presents on the surface of DC and activates the phagocytosis of killed cells by DC. Finally, mature DC will activate tumour cell specific immune responses and therefore it could modify the clinical response of the patient with cancer. Moreover, HP killed cells have easy cryopreservation without affecting their immunogenicity.

This technique is still under development; nevertheless, it is under clinical testings for several cancers indications, such as lung, prostate and ovarian cancers.

Orthopaedics surgery

The actual treatments for exvivo devitalization of infected or tumour affected bone segments are based on irradiation, autoclaving, thermal treatment or use of chemicals. Even if the devitalization is total using these techniques, they reduce the biomechanical and biological integrity of the bone. The procedure is based on the resection, the extracorporeal disinfection and finally the devitalization before its reimplantation.

High pressure can also be used to exvivo devitalization of bone segments without altering the main proteins present in bone tissue (fibronectine, vitronectine and type I

collagen). HP inactivates several bacteria, fungi and virus, however it is necessary to take into account that bacteria in bones are less sensitive to HP than in solution.

For example, HP treatment of 300 MPa for 10 min of a cancerous bone segment is sufficient to inactivate all bone cells, including the bone tumour cells. This possible treatment mainly concerns bone segments and tendons. It has been demonstrated that HP treated Achilles tendons and trabecular bones does not loss their properties after a pressure treatment at 600 MPa. Finally, the revitalization of high pressure treated bone segments has been demonstrated indicating that a successful implementation of HP treated bone segments may be possible.

Moreover, HP may be useful to disinfect biomaterials as prosthesis or bone plates and surgical equipment as screws. It has been demonstrated that a pressure of 300 MPa and 40°C for 30 min disinfect screws contaminated with *Staphylococcus aureus*.

Cartilage stimulus

With the development of tissue engineering, engineered meniscus have been a new option to combat knee cartilage diseases. However, engineered meniscus constructs, without the correct stimulation, possess a lower organization and mechanical properties than the native meniscus.

Cartilage is an avascular and aneural tissue that detects HP, with an adequate stimulation, as a mechanical sign to increase its regeneration. In a normal activity, knee cartilage and synovial joint are hydrostatically pressurized reaching pressures up to 10 MPa. For example, it has been demonstrated that static pressure of 5 MPa for 1.5h increases the glycosaminoglycan incorporation in explants cultures. Interestingly, the effect of pressure on cartilage differs if the study is done on a chondrocytes monolayer or on a three dimensional matrix, which indicates that the interaction cell – extracellular matrix may play a role in HP regulation.

REFERENCES

- [1] Bartlett DH. Microbial Adaptations to the Psychrosphere/Piezosphere. *J Molec Microbiol Biotechnol* 1999;1:93–100.
- [2] Schrenk MO, Huber JA, Edwards KJ. Microbial Provinces in the Subseafloor. *Ann Rev Mar Sci* 2010;2:279–304. doi:10.1146/annurev-marine-120308-081000.
- [3] Hazael R, Meersman F, Ono F, McMillan P. Pressure as a Limiting Factor for Life. *Life* 2016;6:34. doi:10.3390/life6030034.
- [4] Daniel I, Oger P, Winter R. Origins of life and biochemistry under high-pressure conditions. *Chem Soc Rev* 2006;35:858–75. doi:10.1039/b517766a.
- [5] Orcutt BN, Sylvan JB, Knab NJ, Edwards KJ. Microbial Ecology of the Dark Ocean above, at, and below the Seafloor. *Microbiol Mol Biol Rev* 2011;75:361–422. doi:10.1128/MMBR.00039-10.
- [6] Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D’Hondt S. Global distribution of microbial abundance and biomass in subseafloor sediment. *Proc Natl Acad Sci* 2012;109:16213–6. doi:10.1073/pnas.1203849109.
- [7] Oger P, Cario A. La vie sous pression des microorganismes piézophiles. *Biol Aujourd’hui* 2014;208:193–206. doi:10.1051/jbio/2014023.
- [8] Simonato F, Campanaro S, Lauro FM, Vezzi A, D’Angelo M, Vitulo N, et al. Piezophilic adaptation: a genomic point of view. *J Biotechnol* 2006;126:11–25. doi:10.1016/j.jbiotec.2006.03.038.
- [9] Demazeau G, Rivalain N. High hydrostatic pressure and biology: A brief history. *Appl Microbiol Biotechnol* 2011;89:1305–14. doi:10.1007/s00253-010-3070-9.

- [10] Nogi Y, Kato C. Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. *Extremophiles* 1999;3:71–7. doi:10.1007/s007920050101.
- [11] Kusube M, Kyaw TS, Tanikawa K, Chastain RA, Hardy KM, Cameron J, et al. *Colwellia marinimaniae* sp. nov., a hyperpiezophilic species isolated from an amphipod within the challenger deep, Mariana Trench. *Int J Syst Evol Microbiol* 2017;67:824–31. doi:10.1099/ijsem.0.001671.
- [12] Birrien JL, Zeng X, Jebbar M, Cambon-Bonavita MA, Quéréllou J, Oger P, et al. *Pyrococcus yayanosii* sp. nov., an obligate piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* 2011;61:2827–31. doi:10.1099/ijms.0.024653-0.
- [13] Le Chatelier HL. Sur un énoncé général des lois des équilibres chimiques. *Comptes-Rendus l'Académie Des Sci* 1884;99:786–9.
- [14] Mentré P, Hoa GHB. Effects of high hydrostatic pressures on living cells: A consequence of the properties of macromolecules and macromolecule-associated water. *Int Rev Cytol* 2001;201:1–84. doi:10.1016/S0074-7696(01)01001-4.
- [15] Oliveira AC, Gaspar LP, Da Poian AT, Silva JL. Arc repressor will not denature under pressure in the absence of water. *J Mol Biol* 1994;240:184–7. doi:10.1006/jmbi.1994.1433.
- [16] Rayan G, Macgregor RB. Pressure-induced helix-coil transition of DNA copolymers is linked to water activity. *Biophys Chem* 2009;144:62–6. doi:10.1016/j.bpc.2009.06.007.

- [17] Silva JL, Oliveira AC, Vieira TCRG, de Oliveira GAP, Suarez MC, Foguel D. High-Pressure Chemical Biology and Biotechnology. *Chem Rev* 2014;114:7239–67. doi:10.1021/cr400204z.
- [18] Balny C, Masson P, Heremans K. High pressure effects on biological macromolecules: from structural changes to alteration of cellular processes. *Biochim Biophys Acta - Protein Struct Mol Enzymol* 2002;1595:3–10. doi:10.1016/S0167-4838(01)00331-4.
- [19] Balasubramaniam VM (Bala), Martínez-Monteagudo SI, Gupta R. Principles and Application of High Pressure–Based Technologies in the Food Industry. *Annu Rev Food Sci Technol* 2015;6:435–62. doi:10.1146/annurev-food-022814-015539.
- [20] Mentré P, Hoa GHB. Effects of high hydrostatic pressures on living cells: A consequence of the properties of macromolecules and macromolecule-associated water 2001;201:1–84. doi:10.1109/ICCA SM.2010.5619995.
- [21] Rayan G, Macgregor RB. Comparison of the heat- and pressure-induced helix-coil transition of two DNA copolymers. *J Phys Chem B* 2005;109:15558–65. doi:10.1021/jp050899c.
- [22] Takahashi S, Sugimoto N. Effect of pressure on thermal stability of G-Quadruplex DNA and double-stranded DNA structures. *Molecules* 2013;18:13297–319. doi:10.3390/molecules181113297.
- [23] Rayan G, Macgregor RB. Pressure-induced helix–coil transition of DNA copolymers is linked to water activity. *Biophys Chem* 2009;144:62–6. doi:10.1016/J.BPC.2009.06.007.

- [24] Giel-Pietraszuk M, Barciszewski J. A nature of conformational changes of yeast tRNA^{Phe}: High hydrostatic pressure effects. *Int J Biol Macromol* 2005;37:109–14. doi:10.1016/j.ijbiomac.2005.09.003.
- [25] Schuabb C, Berghaus M, Rosin C, Winter R. Exploring the free energy and conformational landscape of tRNA at high temperature and pressure. *ChemPhysChem* 2015;16:138–46. doi:10.1002/cphc.201402676.
- [26] Garcia AE, Paschek D. Simulation of the pressure and temperature folding/unfolding equilibrium of a small RNA hairpin. *J Am Chem Soc* 2008;130:815–7. doi:10.1021/ja074191i.
- [27] Takahashi S, Sugimoto N. Pressure-dependent formation of i-motif and G-quadruplex DNA structures. *Phys Chem Chem Phys* 2015;17:31004–10. doi:10.1039/c5cp04727g.
- [28] Heremans K. Biology under extreme conditions. *High Press Res* 2004;24:57–66. doi:10.1080/08957950310001635828.
- [29] Abe F. Exploration of the Effects of High Hydrostatic Pressure on Microbial Growth, Physiology and Survival: Perspectives from Piezophysiology. *Biosci Biotechnol Biochem* 2007;71:2347–57. doi:10.1271/bbb.70015.
- [30] Panick G, Malessa R, Winter R, Rapp G, Frye KJ, Royer CA. Structural characterization of the pressure-denatured state and unfolding/refolding kinetics of staphylococcal nuclease by synchrotron small-angle X-ray scattering and Fourier-transform infrared spectroscopy. *J Mol Biol* 1998;275:389–402. doi:10.1006/jmbi.1997.1454.
- [31] Marion J, Trovaslet M, Martinez N, Masson P, Schweins R, Nachon F, et al.

Pressure-induced molten globule state of human acetylcholinesterase: Structural and dynamical changes monitored by neutron scattering. *Phys Chem Chem Phys* 2015;17:3157–63. doi:10.1039/c4cp02992e.

[32] Al-Ayoubi SR, Schummel PH, Golub M, Peters J, Winter R. Influence of cosolvents, self-crowding, temperature and pressure on the sub-nanosecond dynamics and folding stability of lysozyme. *Phys Chem Chem Phys* 2017;19:14230–7. doi:10.1039/c7cp00705a.

[33] Roche J, Caro JA, Norberto DR, Barthe P, Roumestand C, Schlessman JL, et al. Cavities determine the pressure unfolding of proteins. *Proc Natl Acad Sci* 2012;109:6945–50. doi:10.1073/pnas.1200915109.

[34] Meersman F, Daniel I, Bartlett DH, Winter R, Hazael R, McMillan PF. High-Pressure Biochemistry and Biophysics. *Rev Mineral Geochemistry* 2013;75:607–48. doi:10.2138/rmg.2013.75.19.

[35] Smeller L. Pressure–temperature phase diagrams of biomolecules. *Biochim Biophys Acta - Protein Struct Mol Enzymol* 2002;1595:11–29. doi:10.1016/S0167-4838(01)00332-6.

[36] Eisenmenger MJ, Reyes-De-Corcuera JI. High pressure enhancement of enzymes: A review. *Enzyme Microb Technol* 2009;45:331–47. doi:10.1016/j.enzmictec.2009.08.001.

[37] Winter R, Dzwolak W. Exploring the temperature-pressure configurational landscape of biomolecules: from lipid membranes to proteins. *Philos Trans Ser A* 2005;363:537–63. doi:10.1098/rsta.2004.1507.

[38] Dutra Albuquerque E, Gonçalves Torres FA, Ribeiro Fernandes AA, Fernandes

PMB. Combined effects of high hydrostatic pressure and specific fungal cellulase improve coconut husk hydrolysis. *Process Biochem* 2016;51:1767–75. doi:10.1016/j.procbio.2016.07.010.

[39] Huang H-W, Lung H-M, Yang BB, Wang C-Y. Responses of microorganisms to high hydrostatic pressure processing. *Food Control* 2014;40:250–9. doi:10.1016/J.FOODCONT.2013.12.007.

[40] Gao M, Berghaus M, Möbitz S, Schuabb V, Erwin N, Herzog M, et al. On the Origin of Microtubules' High-Pressure Sensitivity. *Biophys J* 2018;114:1080–90. doi:10.1016/j.bpj.2018.01.021.

[41] Girard E, Marchal S, Perez J, Finet S, Kahn R, Fourme R, et al. Structure-function perturbation and dissociation of tetrameric urate oxidase by high hydrostatic pressure. *Biophys J* 2010;98:2356–64. doi:10.1016/j.bpj.2010.01.058.

[42] Silva JL, Oliveira AC, Gomes AMO, Lima LMTR, Mohana-Borges R, Pacheco ABF, et al. Pressure induces folding intermediates that are crucial for protein-DNA recognition and virus assembly. *Biochim Biophys Acta - Protein Struct Mol Enzymol* 2002;1595:250–65. doi:10.1016/S0167-4838(01)00348-X.

[43] Luong TQ, Kapoor S, Winter R. Pressure - A Gateway to Fundamental Insights into Protein Solvation, Dynamics, and Function. *ChemPhysChem* 2015;16:3555–71. doi:10.1002/cphc.201500669.

[44] Librizzi F, Carrotta R, Peters J, Cupane A. The effects of pressure on the energy landscape of proteins. *Sci Rep* 2018;8:2037. doi:10.1038/s41598-018-20417-x.

[45] Collins MD, Kim CU, Gruner SM. High-Pressure Protein Crystallography and NMR to Explore Protein Conformations. *Annu Rev Biophys* 2011;40:81–98.

doi:10.1146/annurev-biophys-042910-155304.

- [46] Potekhin SA, Senin AA, Abdurakhmanov NN, Tiktopulo EI. High pressure stabilization of collagen structure. *Biochim Biophys Acta - Proteins Proteomics* 2009;1794:1151–8. doi:10.1016/j.bbapap.2009.04.005.
- [47] Roche J, Ying J, Maltsev AS, Bax A. Impact of Hydrostatic Pressure on an Intrinsically Disordered Protein: A High-Pressure NMR Study of α -Synuclein. *Chembiochem* 2013;14. doi:10.1016/j.dcn.2011.01.002.The.
- [48] Vogel RF, Linke K, Teichert H, Ehrmann MA. High pressure modulated transport and signaling functions of membrane proteins in models and in vivo. *J Phys Conf Ser* 2008;121. doi:10.1088/1742-6596/121/11/112005.
- [49] Macdonald AG, Martinac B. Effect of high hydrostatic pressure on the bacterial mechanosensitive channel MscS. *Eur Biophys J* 2005;34:434–41. doi:10.1007/s00249-005-0478-8.
- [50] Ulmer HM, Herberhold H, Fahsel S, Gänzle MG, Winter R, Vogel RF. Effects of Pressure-Induced Membrane Phase Transitions on Inactivation of HorA, an ATP-Dependent Multidrug Resistance Transporter, in *Lactobacillus plantarum*. *Appl Environ Microbiol* 2002;68:1088–95. doi:10.1042/CS20150223.
- [51] Campanaro S, Vezzi A, Vitulo N, Lauro FM, D'Angelo M, Simonato F, et al. Laterally transferred elements and high pressure adaptation in *Photobacterium profundum* strains. *BMC Genomics* 2005;6:1–15. doi:10.1186/1471-2164-6-122.
- [52] Powalksa E, Jannosch S, Kinne-Saffran E, Kinne RKH, Fontes CFL, Mignaco JA, et al. Fluorescence Spectroscopic Studies of Pressure and Temperature Effects on Na⁺, K⁺-ATPase Reconstituted into Phospholipid Bilayers and

790 Model Raft Mixture Zur Erlangung des akademischen Grades. Biochemistry
791 2007;46:1672–83.

792 [53] Periasamy N, Teichert H, Weise K, Vogel RF, Winter R. Effects of temperature
793 and pressure on the lateral organization of model membranes with functionally
794 reconstituted multidrug transporter LmrA. Biochim Biophys Acta - Biomembr
795 2009;1788:390–401. doi:10.1016/j.bbamem.2008.09.017.

796 [54] Kapoor S, Triola G, Vetter IR, Waldmann H, Winter R. Revealing
797 conformational substates of lipidated N-ras protein by pressure modulation.
798 Biophys J 2012;102:1468. doi:10.1016/j.bpj.2012.02.038.

799 [55] Brooks NJ, Ces O, Templer RH, Seddon JM. Pressure effects on lipid membrane
800 structure and dynamics. Chem Phys Lipids 2011;164:89–98.
801 doi:10.1016/j.chemphyslip.2010.12.002.

802 [56] Matsuki H, Miyazaki E, Sakano F, Tamai N, Kaneshina S. Thermotropic and
803 barotropic phase transitions in bilayer membranes of ether-linked phospholipids
804 with varying alkyl chain lengths. Biochim Biophys Acta - Biomembr
805 2007;1768:479–89. doi:10.1016/j.bbamem.2006.10.005.

806 [57] Winter R, Jeworrek C. Effect of pressure on membranes. Phys B Condens Matter
807 2009;405:2820–6. doi:10.1016/j.physb.2010.04.005.

808 [58] Trapp M, Marion J, Tehei M, Demé B, Gutberlet T, Peters J. High hydrostatic
809 pressure effects investigated by neutron scattering on lipid multilamellar vesicles.
810 Phys Chem Chem Phys 2013;15:20951–6. doi:10.1039/c3cp52762j.

811 [59] Ding W, Palaokostas M, Shahane G, Wang W, Orsi M. Effects of High Pressure
812 on Phospholipid Bilayers. J Phys Chem B 2017;121:9597–606.

doi:10.1021/acs.jpcb.7b07119.

- [60] McCarthy NLC, Ces O, Law R V., Seddon JM, Brooks NJ. Separation of liquid domains in model membranes induced with high hydrostatic pressure. *Chem Commun* 2015;51:8675–8. doi:10.1039/c5cc02134k.
- [61] Golub M, Lehofer B, Martinez N, Ollivier J, Kohlbrecher J, Prassl R, et al. High hydrostatic pressure specifically affects molecular dynamics and shape of low-density lipoprotein particles. *Sci Rep* 2017;7:46034. doi:10.1038/srep46034.
- [62] Lehofer B, Golub M, Kornmueller K, Kriechbaum M, Martinez N, Nagy G, et al. High Hydrostatic Pressure Induces a Lipid Phase Transition and Molecular Rearrangements in Low-Density Lipoprotein Nanoparticles. *Part Part Syst Charact* 2018;35. doi:10.1002/ppsc.201800149.
- [63] Welch TJ, Farewell A, Neighardt FC, Bartlett DH. Stress response in *Escherichia coli* induced by elevated hydrostatic pressure. *J Bacteriol* 1993;175:7170–7.
- [64] Ludwig H. Effects of High Pressure on Bacteria and Funghi. *Adv. High Press. Biosci. Biotechnol.* II, 2003, p. 259–65.
- [65] Frey B, Franz S, Sheriff A, Korn A, Bluemelhuber G, Gaipf US, et al. Hydrostatic pressure induced death of mammalian cells engages pathways related to apoptosis or necrosis. *Cell Mol Biol* 2004;50:459–67. doi:10.1170/T534.
- [66] Oger PM, Jebbar M. The many ways of coping with pressure. *Res Microbiol* 2010;161:799–809. doi:10.1016/j.resmic.2010.09.017.
- [67] Wuytack EY, Diels AMJ, Michiels CW. Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. *Int J Food Microbiol* 2002;77:205–12. doi:10.1016/S0168-1605(02)00054-5.

- [68] Ritz M, Freulet M, Orange N, Federighi M. Effects of high hydrostatic pressure on membrane proteins of *Salmonella typhimurium*. *Int J Food Microbiol* 2000;55:115–9. doi:10.1016/S0168-1605(00)00165-3.
- [69] Pagán R, Mackey B. Relationship between membrane damage and cell death in pressure-treated *Escherichia coli* cells: Differences between exponential- and stationary-phase cells and variation among strains. *Appl Environ Microbiol* 2000;66:2829–34. doi:10.1128/AEM.66.7.2829-2834.2000.
- [70] Patterson MF. Microbiology of pressure-treated foods. *J Appl Microbiol* 2005;98:1400–9. doi:10.1111/j.1365-2672.2005.02564.x.
- [71] Mañas P, Mackey BM. Morphological and Physiological Changes Induced by High Hydrostatic Pressure in Exponential- and Stationary-Phase Cells of *Escherichia coli*: Relationship with Cell Death. *Appl Environ Microbiol* 2004;70:1545–54. doi:10.1128/AEM.70.3.1545-1554.2004.
- [72] Hill C, Cotter PD, Sleator RD, Gahan CGM. Bacterial stress response in *Listeria monocytogenes*: Jumping the hurdles imposed by minimal processing. *Int Dairy J* 2002;12:273–83. doi:10.1016/S0958-6946(01)00125-X.
- [73] Black EP, Setlow P, Hocking AD, Stewart CM, Kelly AL, Hoover DG. Response of spores to high-pressure processing. *Compr Rev Food Sci Food Saf* 2007;6:103–19. doi:10.1111/j.1541-4337.2007.00021.x.
- [74] Michiels C, Bartlett DH, Aertsen A. *High Pressure Microbiology*. vol. ASM Press. 2008.
- [75] Wang CY, Huang HW, Hsu CP, Yang BB. Recent Advances in Food Processing Using High Hydrostatic Pressure Technology. *Crit Rev Food Sci Nutr*

2016;56:527–40. doi:10.1080/10408398.2012.745479.

[76] Jorgensen BB, D'Hondt S. A Starving Majority Deep Beneath the Seafloor.

Science (80-) 2006;314:932–4. doi:10.1126/science.1133796.

[77] Howe A. Deep-sea hydrothermal vent fauna: evolution, dispersal, succession and

biogeography. *Macalester Rev Biogeogr* 2009;1:6.

[78] Orcutt BN, LaRowe DE, Biddle JF, Colwell FS, Glazer BT, Reese BK, et al.

Microbial activity in the marine deep biosphere: Progress and prospects. *Front*

Microbiol 2013;4:1–15. doi:10.3389/fmicb.2013.00189.

[79] Grassle FJ. Hydrothermal Vent Animals: Distribution and Biology. *Science*

(80-) 1985;229:713–7.

[80] Kelly RH, Yancey PH. High contents of trimethylamine oxide correlating with

depth in deep-sea teleost fishes, skates, and decapod crustaceans. *Biol Bull*

1999;196:18–25. doi:10.2307/1543162.

[81] Dover CL Van, German CR, Speer KG, Parson LM, Vrijenhoek RC. Evolution

and Biogeography of Deep-Sea Vent and Seep Invertebrates. *Science* (80-)

2002;295:1253–7. doi:10.1126/science.1067361.

[82] Castellini MA, Castellini JM, Rivera PM. Adaptations to pressure in the RBC

metabolism of diving mammals. *Comp Biochem Physiol - A Mol Integr Physiol*

2001;129:751–7. doi:10.1109/CEIDP.2008.4772920.

[83] Bliznyuk A, Golan H, Grossman Y. Marine Mammals' NMDA Receptor

Structure: Possible Adaptation to High Pressure Environment. *Front Physiol*

2018;9:1–12. doi:10.3389/fphys.2018.01633.

[84] Fang J, Zhang L, Bazylinski DA. Deep-sea piezosphere and piezophiles :

geomicrobiology and biogeochemistry. Trends Microbiol 2010;18:413–22.
doi:10.1016/j.tim.2010.06.006.

[85] Zhang Y, Li X, Xiao X, Bartlett DH. Current developments in marine
microbiology: High-pressure biotechnology and the genetic engineering of
piezophiles. Curr Opin Biotechnol 2015;33:157–64.
doi:10.1016/j.copbio.2015.02.013.

[86] Bright M, Lallier FH. The biology of vestimentiferan tubeworms. Oceanogr Mar
Biol An Annu Rev 2010;48:213–66.

[87] Sobecky PA, Hazen TH. Horizontal Gene Transfer and Mobile Genetic Elements
in Marine Systems. vol. 532. 2009. doi:10.1007/978-1-60327-853-9.

[88] van Wolferen M, Ajon M, Driessen AJM, Albers SV. How hyperthermophiles
adapt to change their lives: DNA exchange in extreme conditions. Extremophiles
2013;17:545–63. doi:10.1007/s00792-013-0552-6.

[89] Li Z, Li X, Xiao X, Xu J. An integrative genomic island affects the adaptations of
the piezophilic hyperthermophilic archaeon *Pyrococcus yayanosii* to high
temperature and high hydrostatic pressure. Front Microbiol 2016;7:1–13.
doi:10.3389/fmicb.2016.01927.

[90] Le Fourn C, Brasseur G, Brochier-Armanet C, Pieulle L, Brioukhanov A,
Ollivier B, et al. An oxygen reduction chain in the hyperthermophilic anaerobe
Thermotoga maritima highlights horizontal gene transfer between
Thermococcales and Thermotogales. Environ Microbiol 2011;13:2132–45.
doi:10.1111/j.1462-2920.2011.02439.x.

[91] Kurosaka G, Abe F. The YPR153W gene is essential for the pressure tolerance of

tryptophan permease Tat2 in the yeast *Saccharomyces cerevisiae*. *High Press Res* 2018;38:90–8. doi:10.1080/08957959.2017.1413367.

[92] Lauro FM, Chastain RA, Blankenship LE, Yayanos AA, Bartlett DH. The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. *Appl Environ Microbiol* 2007;73:838–45. doi:10.1128/AEM.01726-06.

[93] Bidle KA, Bartlett DH. RecD function is required for high-pressure growth of a deep-sea bacterium. *J Bacteriol* 1999;181:2330–7.

[94] Abe F, Kato C, Horikoshi K. Pressure-regulated metabolism in microorganisms. *Trends Microbiol* 1999;7:447–53. doi:10.1016/S0966-842X(99)01608-X.

[95] Li L, Kato C, Nogi Y, Horikoshi K. Distribution of the pressure-regulated operons in deep-sea bacteria. *FEMS Microbiol Lett* 1998;159:159–66. doi:10.1016/S0378-1097(97)00560-0.

[96] Kitahara R, Sareth S, Yamada H, Ohmae E, Gekko K, Akasaka K. High pressure NMR reveals active-site hinge motion of folate-bound *Escherichia coli* dihydrofolate reductase. *Biochemistry* 2000;39:12789–95. doi:10.1021/bi0009993.

[97] Huang Q, Rodgers JM, Hemley RJ, Ichiye T. Extreme biophysics: Enzymes under pressure. *J Comput Chem* 2017;38:1174–82. doi:10.1002/jcc.24737.

[98] Peters J, Martinez N, Michoud G, Cario A, Franzetti B, Oger P, et al. Deep sea microbes probed by incoherent neutron scattering under high hydrostatic pressure. *Zeitschrift Fur Phys Chemie* 2014;228:1121–33. doi:10.1515/zpch-2014-0547.

[99] Martinez N, Michoud G, Cario A, Ollivier J, Franzetti B, Jebbar M, et al. High

protein flexibility and reduced hydration water dynamics are key pressure adaptive strategies in prokaryotes. *Sci Rep* 2016;6:32816. doi:10.1038/srep32816.

[100] Boehr DD, McElheny D, Dyson HJ, Wright PE. Millisecond timescale fluctuations in dihydrofolate reductase are exquisitely sensitive to the bound ligands. *Proc Natl Acad Sci* 2010;107:1373–8. doi:10.1073/pnas.0914163107.

[101] Bartlett DH, Kato C, Horikoshi K. High pressure influences on gene and protein expression. *Res Microbiol* 1995;146:697–706. doi:10.1016/0923-2508(96)81066-7.

[102] Bartlett DH. Pressure effects on in vivo microbial processes. *Biochim Biophys Acta* 1595 2002;1595:367–81. doi:10.1002/smj.

[103] Feller G. Psychrophilic Enzymes: From Folding to Function and Biotechnology. *Scientifica (Cairo)* 2013;2013:1–28. doi:10.1155/2013/512840.

[104] Ohmae E, Murakami C, Tate SI, Gekko K, Hata K, Akasaka K, et al. Pressure dependence of activity and stability of dihydrofolate reductases of the deep-sea bacterium *Moritella profunda* and *Escherichia coli*. *Biochim Biophys Acta - Proteins Proteomics* 2012;1824:511–9. doi:10.1016/j.bbapap.2012.01.001.

[105] Ichiye T. Enzymes from piezophiles. *Semin Cell Dev Biol* 2018;84:138–46. doi:10.1016/j.semcdb.2018.01.004.

[106] Sonavane S, Chakrabarti P. Cavities and atomic packing in protein structures and interfaces. *PLoS Comput Biol* 2008;4. doi:10.1371/journal.pcbi.1000188.

[107] Collins MD, Hummer G, Quillin ML, Matthews BW, Gruner SM. Cooperative water filling of a nonpolar protein cavity observed by high-pressure

crystallography and simulation. Proc Natl Acad Sci 2005;102:16668–71.
doi:10.1073/pnas.0508224102.

[108] Rosenbaum E, Gabel F, Durá MA, Finet S, Cléry-Barraud C, Masson P, et al.
Effects of hydrostatic pressure on the quaternary structure and enzymatic activity
of a large peptidase complex from *Pyrococcus horikoshii*. Arch Biochem
Biophys 2012;517:104–10. doi:10.1016/j.abb.2011.07.017.

[109] Jebbar M, Franzetti B, Girard E, Oger P. Microbial diversity and adaptation to
high hydrostatic pressure in deep-sea hydrothermal vents prokaryotes.
Extremophiles 2015;19:721–40. doi:10.1007/s00792-015-0760-3.

[110] Yancey PH, Blake WR, Conley J. Unusual organic osmolytes in deep-sea
animals: Adaptations to hydrostatic pressure and other perturbants. Comp
Biochem Physiol - A Mol Integr Physiol 2002;133:667–76. doi:10.1016/S1095-
6433(02)00182-4.

[111] Cario A, Jebbar M, Thiel A, Kervarec N, Oger PM. Molecular chaperone
accumulation as a function of stress evidences adaptation to high hydrostatic
pressure in the piezophilic archaeon *Thermococcus barophilus*. Sci Rep
2016;6:1–8. doi:10.1038/srep29483.

[112] Brown A, Thatje S. NMDA receptor regulation is involved in the limitation of
physiological tolerance to both low temperature and high hydrostatic pressure.
Front Mar Sci 2018;5:1–4. doi:10.3389/fmars.2018.00093.

[113] Sinensky M. Homeoviscous Adaptation-A Homeostatic Process that Regulates
the Viscosity of Membrane Lipids in *Escherichia coli*. Proc Natl Acad Sci
1974;71:522–5. doi:10.1073/pnas.71.2.522.

- [114] Van de Vossenberg JLCM, Driessen AJM, Konings WN. The essence of being extremophilic: The role of the unique archaeal membrane lipids. *Extremophiles* 1998;2:163–70. doi:10.1007/s007920050056.
- [115] Oger PM, Cario A. Adaptation of the membrane in Archaea. *Biophys Chem* 2013;183:42–56. doi:10.1016/j.bpc.2013.06.020.
- [116] Siliakus MF, van der Oost J, Kengen SWM. Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. *Extremophiles* 2017;21:651–70. doi:10.1007/s00792-017-0939-x.
- [117] Usui K, Hiraki T, Kawamoto J, Kurihara T, Nogi Y, Kato C, et al. Eicosapentaenoic acid plays a role in stabilizing dynamic membrane structure in the deep-sea piezophile *Shewanella violacea*: A study employing high-pressure time-resolved fluorescence anisotropy measurement. *Biochim Biophys Acta - Biomembr* 2012;1818:574–83. doi:10.1016/j.bbamem.2011.10.010.
- [118] Myka K, Allcock DJ, Eloë-Fadrosh EA, Tryfona T, Haag AF, Lauro FM, et al. Adaptations of Cold- and Pressure-Loving Bacteria to the Deep-Sea Environment: Cell Envelope and Flagella. *Microb Ecol Extrem Environ* 2017;1–245. doi:10.1007/978-3-319-51686-8.
- [119] Kawamoto J, Sato T, Nakasone K, Kato C, Mihara H, Esaki N, et al. Favourable effects of eicosapentaenoic acid on the late step of the cell division in a piezophilic bacterium, *Shewanella violacea* DSS12, at high-hydrostatic pressures. *Environ Microbiol* 2011;13:2293–8. doi:10.1111/j.1462-2920.2011.02487.x.
- [120] Kaneshiro SM, Clark DS. Pressure Effects on the Composition and Thermal-Behavior of Lipids From the Deep-Sea Thermophile *Methanococcus-Jannaschii*. *J Bacteriol* 1995;177:3668–72. doi:10.1128/jb.177.13.3668-3672.1995.

- [121] Cario A, Grossi V, Schaeffer P, Oger PM. Membrane homeoviscous adaptation in the piezo-hyperthermophilic archaeon *Thermococcus barophilus*. *Front Microbiol* 2015;6:1–12. doi:10.3389/fmicb.2015.01152.
- [122] Hradilova N, Sadilkova L, Palata O, Mysikova D, Mrazkova H, Lischke R, et al. Generation of dendritic cell-based vaccine using high hydrostatic pressure for non-small cell lung cancer immunotherapy. *PLoS One* 2017;12:1–18. doi:10.1371/journal.pone.0171539.
- [123] Adkins I, Hradilova N, Palata O, Sadilkova L, Palova-Jelinkova L, Spisek R. High hydrostatic pressure in cancer immunotherapy and biomedicine. *Biotechnol Adv* 2018;36:577–82. doi:10.1016/j.biotechadv.2018.01.015.
- [124] Ferreira E, Mendes YS, Silva JL, Galler R, Oliveira AC, Freire MS, et al. Effects of hydrostatic pressure on the stability and thermostability of poliovirus: A new method for vaccine preservation. *Vaccine* 2009;27:5332–7. doi:10.1016/j.vaccine.2009.06.099.
- [125] Gu R, Feng Y, Guo S, Zhao S, Lu X, Fu J, et al. Improved cryotolerance and developmental competence of human oocytes matured in vitro by transient hydrostatic pressure treatment prior to vitrification. *Cryobiology* 2017;75:144–50. doi:10.1016/j.cryobiol.2016.12.009.
- [126] Brouillet M, Gautier H, Miègeville AF, Bouler JM, Merle C, Caillon J. Inactivation of *Staphylococcus aureus* in calcium phosphate biomaterials via isostatic compression. *J Biomed Mater Res - Part B Appl Biomater* 2009;91:348–53. doi:10.1002/jbm.b.31408.
- [127] Huang HW, Wu SJ, Lu JK, Shyu YT, Wang CY. Current status and future trends of high-pressure processing in food industry. *Food Control* 2017;72:1–8.

- doi:10.1016/j.foodcont.2016.07.019.
- [128] Farkas DF, Hoover DG. High pressure processing. *J Food Sci* 2000;65:47–64.
- [129] Polydera AC, Stoforos NG, Taoukis PS. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *J Food Eng* 2003;60:21–9. doi:10.1016/S0260-8774(03)00006-2.
- [130] Bull MK, Zerdin K, Howe E, Goicoechea D, Paramanandhan P, Stockman R, et al. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov Food Sci Emerg Technol* 2004;5:135–49. doi:10.1016/j.ifset.2003.11.005.
- [131] Kaur BP, Kaushik N, Rao PS, Mishra HN. Chilled storage of high pressure processed black tiger shrimp (*Penaeus monodon*). *J Aquat Food Prod Technol* 2015;24:283–99. doi:10.1080/10498850.2013.772271.
- [132] Evert-Arriagada K, Hernández-Herrero MM, Juan B, Guamis B, Trujillo AJ. Effect of high pressure on fresh cheese shelf-life. *J Food Eng* 2012;110:248–53. doi:10.1016/j.jfoodeng.2011.05.011.
- [133] Sazonova S, Galoburda R, Gramatina I. Application of high-pressure processing for safety and shelf-life quality of meat- a review. *11th Balt Conf Food Sci Technol “FOODBALT 2017”* 2017:17–22.
- [134] Ananta E, Knorr D. Evidence on the role of protein biosynthesis in the induction of heat tolerance of *Lactobacillus rhamnosus* GG by pressure pre-treatment. *Int J Food Microbiol* 2004;96:307–13. doi:10.1016/j.ijfoodmicro.2004.04.012.
- [135] Zhao S, Baik OD, Choi YJ, Kim SM. Pretreatments for the Efficient Extraction of Bioactive Compounds from Plant-Based Biomaterials. *Crit Rev Food Sci Nutr*

1045 2014;54:1283–97. doi:10.1080/10408398.2011.632698.

1046 [136] Vanga SK, Singh A, Raghavan V. Review of conventional and novel food
 1047 processing methods on food allergens. *Crit Rev Food Sci Nutr* 2017;57:2077–94.
 1048 doi:10.1080/10408398.2015.1045965.

1049 [137] Zhou H, Wang C, Ye J, Tao R, Chen H, Li W, et al. Improvement of allergenicity
 1050 and functional properties of proteins from ginkgo seeds by high hydrostatic
 1051 pressure treatment. *Nongye Gongcheng Xuebao/Transactions Chinese Soc Agric*
 1052 *Eng* 2016;32:292–8. doi:10.11975/j.issn.1002-6819.2016.08.041.

1053 [138] Kim MY, Lee SH, Jang GY, Park HJ, Li M, Kim S, et al. Effects of high
 1054 hydrostatic pressure treatment on the enhancement of functional components of
 1055 germinated rough rice (*Oryza sativa* L.). *Food Chem* 2015;166:86–92.
 1056 doi:10.1016/j.foodchem.2014.05.150.

1057 [139] Peñas E, Gomez R, Frias J, Baeza ML, Vidal-Valverde C. High hydrostatic
 1058 pressure effects on immunoreactivity and nutritional quality of soybean products.
 1059 *Food Chem* 2011;125:423–9. doi:10.1016/j.foodchem.2010.09.023.

1060 [140] Li Y, Yang W, Chung SY, Chen H, Ye M, Teixeira AA, et al. Effect of Pulsed
 1061 Ultraviolet Light and High Hydrostatic Pressure on the Antigenicity of Almond
 1062 Protein Extracts. *Food Bioprocess Technol* 2013;6:431–40. doi:10.1007/s11947-
 1063 011-0666-8.

1064 [141] Houska M, Heroldova M, Vavrova H, Kucera P, Setinova I, Havranova M, et al.
 1065 Is high-pressure treatment able to modify the allergenicity of the main apple juice
 1066 allergen, Mal d1? *High Press Res* 2009;29:14–22.
 1067 doi:10.1080/08957950802454068.

- [142] Ahammer L, Grutsch S, Kamenik AS, Liedl KR, Tollinger M. Structure of the Major Apple Allergen Mal d 1. *J Agric Food Chem* 2017;65:1606–12. doi:10.1021/acs.jafc.6b05752.
- [143] Chicón R, Belloque J, Alonso E, Mart N-Lvarez PJ, Pez-Fandi ARL. Hydrolysis under High Hydrostatic Pressure as a Means To Reduce the Binding of β -Lactoglobulin to Immunoglobulin E from Human Sera. *J Food Prot* 2008;71:1453–9. doi:10.4315/0362-028X-71.7.1453.
- [144] Oliveira AC, Ishimaru D, Gonçalves RB, Smith TJ, Mason P, Sá-Carvalho D, et al. Low temperature and pressure stability of picornaviruses: Implications for virus uncoating. *Biophys J* 1999;76:1270–9. doi:10.1016/S0006-3495(99)77290-5.
- [145] Silva JL, Foguel D, Poian AT Da, Prevelige PE. The use of hydrostatic pressure as a tool to study viruses and other macromolecular assemblages. *Curr Opin Struct Biol* 1996;6:166–75. doi:10.1002/aic.690140505.
- [146] Dumard CH, Barroso SPC, Santos AC V., Alves NS, Couceiro JNSS, Gomes AMO, et al. Stability of different influenza subtypes: How can high hydrostatic pressure be a useful tool for vaccine development? *Biophys Chem* 2017;231:116–24. doi:10.1016/j.bpc.2017.04.002.
- [147] Silva JL, Luan P, Glaser M, Voss EW, Weber G. Effects of hydrostatic pressure on a membrane-enveloped virus: high immunogenicity of the pressure-inactivated virus. *J Virol* 1992;66:2111–7.
- [148] Lemay P. The use of high pressure for separation and production of bioactive molecules. *Biochim Biophys Acta - Protein Struct Mol Enzymol* 2002;1595:357–66. doi:10.1016/S0167-4838(01)00356-9.

- [149] Estevez-Burugorri L, Degraeve P, Espeillac S, Lemay P. High-pressure induced recovery of β -galactosidases from immunoabsorbents: Stability of antigens and antibodies. Comparison with usual elution procedures. *Biotechnol Lett* 2000;22:1319–29. doi:10.1023/A:1005659215110.
- [150] Cheung CY, Green DJ, Litt GJ, Laugharn JA. High-pressure-mediated dissociation of immune complexes demonstrated in model systems. *Clin Chem* 1998;44:299–303.
- [151] Niemeyer B, Jansen J. An innovative approach for sorptive separation of amphiphilic biomolecules applying high hydrostatic pressure. *J Supercrit Fluids* 2007;39:354–61. doi:10.1016/j.supflu.2006.03.015.
- [152] Chura-Chambi RM, Cordeiro Y, Malavasi N V., Lemke LS, Rodrigues D, Morganti L. An analysis of the factors that affect the dissociation of inclusion bodies and the refolding of endostatin under high pressure. *Process Biochem* 2013;48:250–9. doi:10.1016/j.procbio.2012.12.017.
- [153] Picard A, Daniel I, Montagnac G, Oger P. In situ monitoring by quantitative Raman spectroscopy of alcoholic fermentation by *Saccharomyces cerevisiae* under high pressure. *Extremophiles* 2007;11:445–52. doi:10.1007/s00792-006-0054-x.
- [154] Bothun GD, Knutson BL, Berberich JA, Strobel HJ, Nokes SE. Metabolic selectivity and growth of *Clostridium thermocellum* in continuous culture under elevated hydrostatic pressure. *Appl Microbiol Biotechnol* 2004;65:149–57. doi:10.1007/s00253-004-1554-1.
- [155] Mota MJ, Lopes RP, Delgadillo I, Saraiva JA. Probiotic yogurt production under high pressure and the possible use of pressure as an on/off switch to stop/start

1116 fermentation. *Process Biochem* 2015;50:906–11.
 1117 doi:10.1016/j.procbio.2015.03.016.

1118 [156] Mota MJ, Lopes RP, Delgadillo I, Saraiva JA. Microorganisms under high
 1119 pressure - Adaptation, growth and biotechnological potential. *Biotechnol Adv*
 1120 2013;31:1426–34. doi:10.1016/j.biotechadv.2013.06.007.

1121 [157] Dumorné K, Córdova DC, Astorga-Eló M, Renganathan P. Extremozymes: A
 1122 potential source for industrial applications. *J Microbiol Biotechnol* 2017;27:649–
 1123 59. doi:10.4014/jmb.1611.11006.

1124 [158] Schroeder G, Bates SS, La Barre S. Bioactive Marine Molecules and Derivatives
 1125 with Biopharmaceutical Potential. *Blue Biotechnol* 2018:611–41.
 1126 doi:10.1002/9783527801718.ch19.

1127 [159] Dalmaso GZL, Ferreira D, Vermelho AB. Marine extremophiles a source of
 1128 hydrolases for biotechnological applications. *Mar Drugs* 2015;13:1925–65.
 1129 doi:10.3390/md13041925.

1130 [160] Benvegna T, Lemiègre L, Cammas-marion S. New Generation of Liposomes
 1131 Called Archaeosomes Based on Natural or Synthetic Archaeal Lipids as
 1132 Innovative Formulations for Drug Delivery. *Recent Pat Drug Deliv Formul*
 1133 2009;33:206–20. doi:10.2174/187221109789105630.

1134 [161] Jacobsen AC, Jensen SM, Fricker G, Brandl M, Treusch AH. Archaeal lipids in
 1135 oral delivery of therapeutic peptides. *Eur J Pharm Sci* 2017;108:101–10.
 1136 doi:10.1016/j.ejps.2016.12.036.

1137 [162] Tortorella E, Tedesco P, Palma Esposito F, January GG, Fani R, Jaspars M, et al.
 1138 Antibiotics from Deep-Sea Microorganisms: Current Discoveries and

1139 Perspectives. Mar Drugs 2018;16:1–16. doi:10.3390/md16100355.

1140 [163] Janakiram NB, Mohammed A, Rao C V. Sea cucumbers metabolites as potent

1141 anti-cancer agents. Mar Drugs 2015;13:2909–23. doi:10.3390/md13052909.

1142