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Applications of High Pressure in Biological Sciences and Food Technology

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Abstract

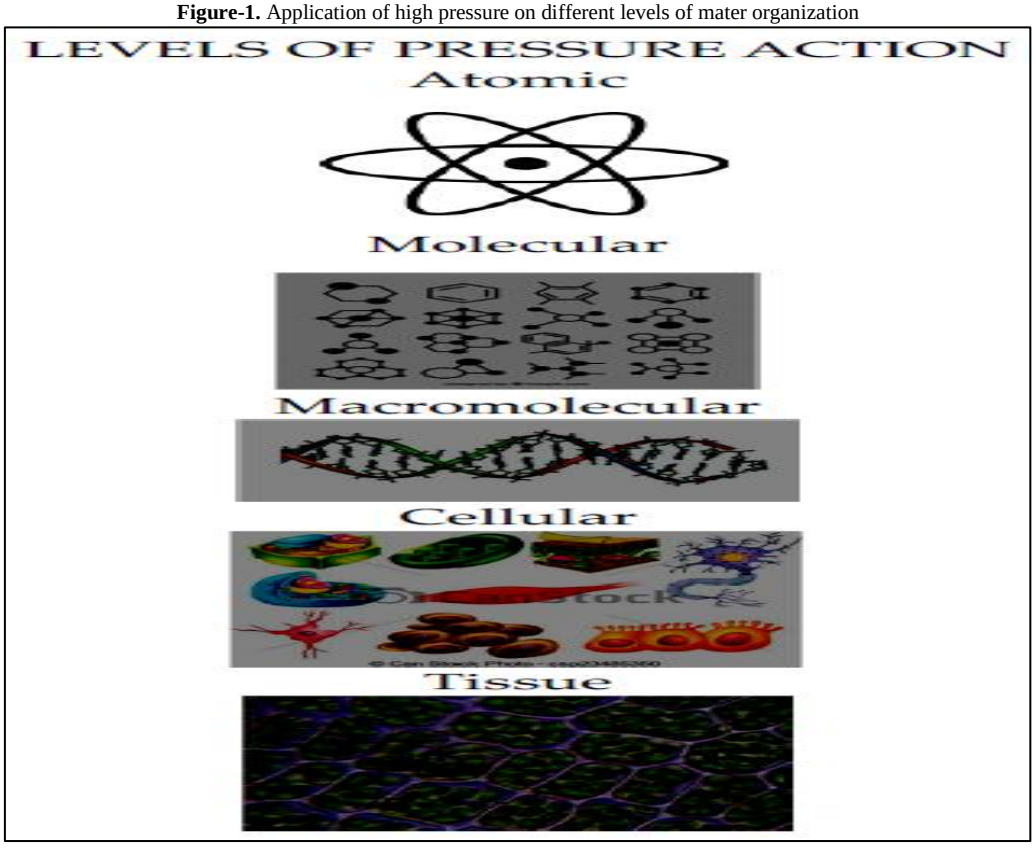
The aim of this review is the overview the main applications of high hydrostatic pressure in science, especially in the biological sciences such as: biochemistry, biology, food technology or medicine. In this work will be shown the impact of high pressure on the biochemical processes and on living systems. The application of pressure on food preparation and sterilization will be shown. We will be paying close of the chemical Le Chetelier's rule as the fundamental principle for understanding the role of pressure in biology and biochemistry. The unsupported area principle will be explained as the universal basis for all pressure used techniques. The basic piston cylinder technique used to produce pressures also refers to the biology applications will be shown.

Keywords: Hydrostatic pressure; Le chetelier rule; Unsupported area principle; Pressure efects biology and biochemistry; Technology based on pressure.

1. Introduction

Pressure substantially impacts on the structure, nature and properties of matter both non-living and living ones. Pressure acts on different levels of organization of matter, from atomic, molecular, macromolecular, cellular, and tissue levels (Figure 1). Generally the pressure affects on the nature of whole our earth and cosmos including all living systems.

Figure-1. Application of high pressure on different levels of mater organization



High pressure reduces a distance between particles and changes the properties of many materials including biological ones. It allows improving the structure of some materials in many branches of technology. It refers to the

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food, industry, geophysics, biology and medicine. Particular importance is the application of pressure in food technology. Pressure sterilization process called pressurization replaces temperature sterilization. Pressure can inactivae some enzymes and in this manner inactivates many harmful microorganisms.

In laboratories, high pressure is used the study the nature matter, especially on the quantum level. Pressure affects on the kinetic of chemical and biochemical reactions. In biochemistry, high pressure has significant effects on biological macromolecules, such as proteins, lipids and sacharides. Pressure affects also on the living organisms, both terrestrial and aquatic.

2. Main Rules and Devices used to Pressure Studies

2.1. Le Chetelier rule

Chemical Le Chetelier rule is the fundamental principle for understanding the role of pressure in biology and biochemistry. The le Chatelier rule maintains a thermodynamic equilibrium in the system as it tends to minimize the effects of disturbing factors of equilibrium. In the chemical and biochemical systems, increased pressure favors the reduction in the volume as to maintain thermodynamic equilibrium:

$$\left(\frac{\partial \ln K}{\partial P}\right)_T = -\frac{\Delta V}{RT} \qquad (1)$$

where:

K- equilibrium constant,

P –pressure,

ΔV –volume change,

R – gas constant,

T- absolute temperature

According to the Le Chatelier rule an increase in pressure favors reduction of the volume of a system.

For an elementary equilibrium process A → B, we have the following general thermodynamic expression:

$$\Delta G = -RT \ln K = \Delta E + p\,\Delta V - T\Delta S \qquad (2)$$

where ΔG, ΔE, ΔV and ΔS are the changes in free energy, internal energy, volume, and entropy; K is the equilibrium constant governing the process, T - the temperature, p -the pressure, and R - the gas constant.

As an example of Le Chatelier rule action, we can include an EPR study lysosyme [1]. The EPR spectra show that as pressure increases the population of the immobilized state increases which is manifested in a decrease in volume of the molecule. The linear dependence of ln (KP/K0) on pressure is observed on plot for lysosyme. Pressure favors the interaction with the neighboring glutamate residue because of a smaller molar volume of the complex. KP and K0 are the equilibrium constants at pressure P and 0.1 MPa, respectively.

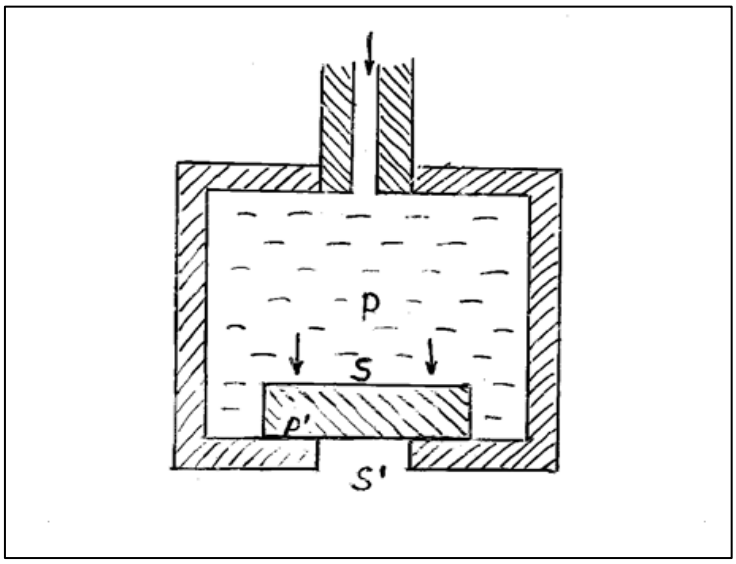
Van't Hoff (in 1901) and Evans and Polanyi (in 1935) proposed that equation (1) may also apply to a constant rate of chemical reaction. Hence the influence of pressure on the rate constant of a given reaction is determined.

The pressure reduces the distances between atoms by reducing the volume of the system. When the pressure increases, the volume decreases. For using to biological systems has limited use – pressure not may be too high.

2.2. Unsupported Area Principle

The unsupported area principle, introduced by Bridgman [2] is the most important principle in the all high pressure techniques. The idea of unsupported area seal illustrates the Figure 2. p denotes pressure inside and p' denotes the pressure on the gasket, S – supported area, S'-unsupported area. The unsupported area S' makes the pressure on the gasket p' is larger than inside the chamber p: p'>p. It follows from the following principle:

Figure-2. Illustrated by the principle of unsupported area proposed by Bridgman: p - pressure inside, p' - the pressure on the gasket, S – supported area, S'-unsupported area



$$pS = p'(S - S') \tag{3}$$

whence it follows that:

$$p' = p\frac{S}{S - S'} \tag{4}$$

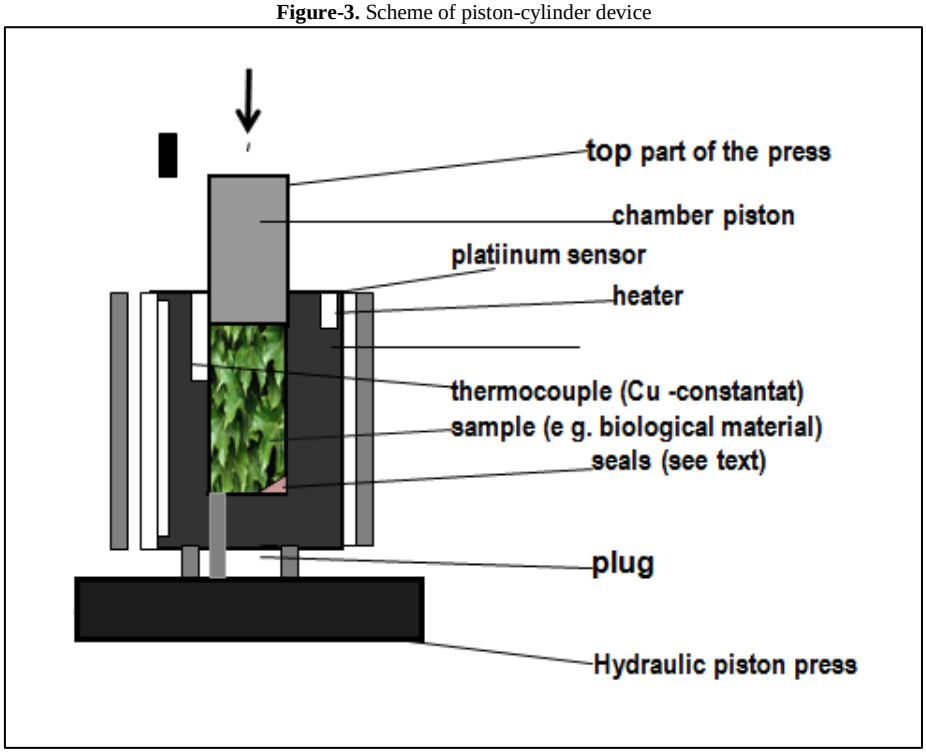
hence:

$$p' > p \tag{4a}$$

2.3. Main Device

Piston-cylinder devices are widely applied in the high-pressure techniques (Figure 3) also for testing biological or biochemical material eg. starch and others. This device consists of a cylinder and a piston made of hardened steel. Steel for use in the high-pressure pistons and cylinders should have a maximum compressive strength. Steel toughens up for obtaining hardness 62-64 HRC. Steel ŁH15 is the most popular and often used. For the constructions of high-pressure cylinders materials such as stainless steels and titanium alloys are used.

Figure-3. Scheme of piston-cylinder device



The inside pressure of the cylinder is determined from the force acting on the piston. The sealing of the narrow aperture between the chamber and the movement of the piston as well as the plug is ensured by a precise fit to the inside of the chamber of the low-pressure rubber o-ring. For pressures greater than 2 MPa, copper or brass anti-extrusion rings of a right triangle shoud be used , as shown schematically in Fig. 3. Outside the chamber there is a heat exchanger that allows you to control the temperature of the system from 96 K to around 400 K by passing through a vapor of nitrogen and heating up the temperature control, which is connected to a platinum sensor placed on the chamber (Fig. 3) to the electric burner directly on the chamber. The temperature is measured by a thermocouple placed in the narrow channel of the chamber. In biology, maximum pressures up to 600 MPa are used.

3. High Pressure in Biology Biochemistry and Medicine

The range of pressure for the biosphere is fairly significant: the peaks of the Himalayas to the Marian trench [3]. Pressure has a significant impact on a number of live organisms. Pressure affects at different levels of biological organization: from molecular through cellular and structural level (Figure 1). Most of studies of pressure impact on living systems take place in vitro.

This range of pressure applies especially to the organisms living in the see. Pressure has a significant impact on growth and survival on the mid-water and bentonithic organisms and might significantly influence larvae colonization patterns and adult distributions in the deep-sea [4, 5] as well as plays a significant role in the early studies in embryos and effects on eggs, cells, and embryos [6-8]. At the deepest point in the ocean, slightly under 11000 meters, the perceptible pressure on our body would be 1100 times greater than what you experience in the open air. The formula that gives the pressure p on an object submerged in a fluid is:

$$p = \rho g h \tag{4}$$

where

ρ - is the density of the fluid,

g - is the acceleration of gravity

h - is the height of the fluid above the object

The tolerance range for pressure values is different for different species. Temperature and pressure tolerance of embryos and larvae of the sea urchin genus *Echinus* has been studied [4]. The Marianas Trench s the deepest part of the world's oceans. At the bottom of the trench the exerts a pressure of 1086 bars more than 1000 times the standard atmospheric pressure at sea level. In the Marian Trench, where there is enormous pressure, several living species have been discovered recently. According to BBC sources there are some examples. In July 2011 some gigantic single-celled amoebas with a size of more than 10 cm belonging to the class of xenophyophores were observed. In December 2014, a new species of snailfish was discovered at a depth of 8,145 m. During the 2014 expedition, several new species were filmed including huge crustaceans known as supergiants. In May 2017 an unidentified type of snailfish was filmed at a depth of 8,178 metres. For creatures like the beaked whale, they have a wide range of adaptations to accommodate their deep-sea lifestyle choices. The lungs of these creatures are completely compressible and organs have adapted to hold more myoglobin and hemoglobin. The pressure there is very high and yet organisms live there. This proves a huge adaptation of life to such pressure conditions.

Could life exist at very low pressures, such as prevailing in space? Recently growth of *Bacillus subtilis* cells, normally adapted at Earth-normal atmospheric pressure (101.3 kPa), was progressively inhibited by lowering of pressure in liquid LB medium until growth essentially ceased at 2.5 kPa or 5 kPa. From these studies a generation population was obtained that showed an increase in fitness at 5 kPa [9].

Pressure effect has an important role in the biomedicine [10-12].The pressures on human articular cartilage have been measured in vivo [10]. The chondrocytes appear to react to the changes in hydrostatic pressure [12] Pure hydrostatic pressure itself affects the proliferation of cultured rat mesangial cells [11]. Negative pressure i.e. below atmospheric pressure is used in the following therapies: removal of inflammatory changes, as a relaxation treatment and detoxification, to increase the blood supply to internal organs as well as skin and subcutaneous tissue, to relax all kinds of fascial adhesions, for the treatment of muscle pain.

Suitable pressure affects the proper action of proteins, enzymes, reaction rate constants, thermodynamic equilibrium in the living systems [13-15].

Pressure has effects on expression of genes [16-18].Most of the studies of pressure impact on gene expression take places *in vitro*. A regulatory DNA element upstream of the pressure-regulated operon from deep-sea *Shewanella* strain DSS12 was studied. The results obtained indicate that the deep-sea strain DSS12 expresses different DNA-binding factors under different pressure conditions. Nakasone, *et al.* [17]. It was shown that exposure of HeLa S3 cells to high hydrostatic pressure 6.89 x $l0^3$ to 6.89x$l0^4$ kPa reduced core and HI histone mRNA levels. At 4.14x10^4 kPa for 10 min core histone and HI histone mRNA levels were reduced 32-38% and 58%, respectively. At 4.14x10^4 kPa for 15min, there was a 42% reduction in core histone mRNA [19]. The pressures on human articular cartilage have been measured in vivo [10].

High pressure has significant effects on biological macromolecules, such as proteins, lipids, sacharides and many cellular processes [14]. Experimentally, it has been observed that increase in hydrostatic pressure can both increase and decrease protein stability. Volume changes upon protein unfolding can be both positive and negative. No effect of pressure occurs in the primary protein structure. Effect of pressure is visible in the secondary, tertiary, quaternary protein structure. Kundrot and Richards' found that different regions in the lysozyme molecule are compressible to different extents. They found that deformation of □□sheet regions is smaller than that of□□-helices [20]. High pressure has the significant important property of stabilizing partially folded states or molten-globule states of a protein. This may impact on some health problems [20]. Pressure induced effect of DNA binding on stabilization of LexA dimers were observed. Pressure provides convenient means to study protein conformational changes. It is particularly applicable to the study of protein aggregation [20] and/or amyloid fibril formation. Pressure can inactivate some enzyme without any change in protein structure [15]. Pressure can stabilize enzymes and modulate both their activity and specificity. The effect of high hydrostatic pressure (HHP) on the rate and equilibrium state of intracellular reactions has been studied [21] In *Escherichia coli* the effect of changes in the ribosome conformation has been studied *in vivo* using DSC technique [22]. In pressure-treated cells ribosomes had adopted a less stable conformation. It is well known that proteins denature under high pressure.

High pressure impacts on protein structure for deep-sea animals [23]. Hydrostatic pressure inhibits many protein function involving positive changes. Some see protein evolved adapting to the pressure prevailing in the depths. Adenosine receptor − inhibitory G protein (Gi) − adenylyl cyclase signaling complex was examined in brain membrane preparations from four teleost fish species of the deep-sea family Macrouridae [24]. Basal adenylyl cyclase activity, determined at 5°C, was inhibited by increased hydrostatic pressure in all four species. At the highest pressure tested, 476 atm, adenylyl cyclase activity was inhibited from 60% to 70% relative to the atmospheric pressure values. The responsiveness of adenylyl cyclase activity to modulation by N6-cyclopentyladenosine, an A1adenosine receptor agonist, was retained at elevated pressures. The effect of hydrostatic pressure in the range 0.1–54·MPa, equivalent to pressures experienced by fish from the ocean's surface to depths of ca. 5400·m on visual pigment absorption spectra was investigated for rod visual pigments extracted from the retinae of 12 species of deepsea fish of diverse phylogeny and habitat [25]. The wavelength of peak absorption (max) was shifted to longer wavelengths by an average of 1.35·nm at 40·MPa compared to atmospheric pressure 0.1 MPa.

The pressure may increase immunogenic properties of pressure treated proteins, killed viruses and microorganisms [26]. High pressure causes inactivation of vegetative microorganisms [27].

There are various methods of high-pressure studies of protein structure such as: vibrational spectroscopy [18], NMR spectroscopy [16], X-ray analysis [28], UV-vis and fluorescence spectroscopy [20], Molecular dynamic

simulation [29], flash photolysis [30]. Pressure effects on enzyme actvity effects studied by different spectroscopies [20], stopped flow or rapid sampling techniques [31, 32].

4. Application of Pressure in Food

Pressure is also used as a tool for the preparation and maintenance of the food. High- pressure processing (HPP) is a cold pasteurization technique. This technique dates back to the 19th century. The first high pressure food processing equipment was used to pressurize milk by Hite in 1899 [33].

High pressure processing, called pressurization is a process that helps maintain the fresh food characteristics like flavours and nutrients. It is an alternative technique to traditional thermal and chemical ones. High pressure processing can be conducted at ambient or refrigerated temperatures.

Foods which receive HPP must be first pre-packed in vacuum-packs and then are placed into a specially designed pressure chamber which is sealed and completely filled with water. A pump connected to the pressure chamber pressurises the water, i.e. hydrostatic pressure, and this pressure is then transmitted to the food through its packaging.The pressure is then applied for a certain time, usually from a few seconds to 20 minutes. In most processing operations HPP is carried out between 400 to 600 MPa at room temperature.

High pressure applications in food technology are as follows:

- reducing the foods microbial load,
- elimination or reduction to safe levels pathogens of concern such as *Listeria monocytogenes*,
- for enhancing the characteristics of reformulated products,
- can preserve or improve the organoleptic properties of food,
- product forming,
- can be used for shucking shellfish and other seafood,
- others.

The high hydrostatic pressure (HHP) to a food product may kill many microorganisms. Pressure pasteurization kills vegetative bacteria. Let consider some examples. Many pathogens are inactivated by HPP. The mechanism and kinetics of pressure-induced degradation/denaturation/(in)activation of several food compounds (e.g. microorganisms, enzymes, nutrients) was considered [34]. HPP can be used to process both liquid and solid foods. HPP technology applies especially for foods with a high acid content. High pressure processing inactivates *Salmonella*, *E. coli* and *Listeria monocytogenes* in fruit and vegetable products. HPP is especially important to the meat processors producing sliced deli meats, because prevent from the risk of re-contamination with harmful pathogens. Very high pressure up to 600 MPa, lasting less than a minute cause in juices and beverages the inactivation of spoilage organisms, including yeast and mould, and harmful pathogens, as well as the reduction of enzymatic activity. High pressure technology gives food manufacturers the tools to provide safer more natural products with extended quality and shelf-life. In see-food high pressure processing enables 100% separation from lobsters, oysters, clams, and other fresh products by denaturing the specific protein that holds the meat to the shell. The high pressure applies whey protein denaturation and cheese-making property of raw milk is described [35].

High pressure treatment promotes lipids oxidation in fish oil-in-water emulsion systems [36]. Several tomato samples have been undergone under pressure (up to 600 MPa) [37]. The results showed that the texture and tissue not be damaged by high pressure in the range at 400 MPa for 20 min and or 500 MPa for 5 min. The pressure allows freezing, thawing and storage of food at subzero temperature without freezing [38]. Theory and practice of high pressure freezing is described in the book [39]. Pressurized temperature denaturation of the protein graph shows that the pressure can replace cooking, e.g. egg. Pressure cooked egg retains the flavor of raw eggs. Under the influence of pressure there are no chemical processes. Boiled egg loses flavor because there is migration of sulfur compounds from the protein to yolk [40]

High pressure may influence on the number of free radicals in foods. In the phosphorylated potato starch [41] and in the phosphorylated maize starch [42] decrease in free radicals after application of high hydrostatic pressure have been observed. Our study of potato starch showed that pressurization time in a hydrostatic press up to 1000 MPa has a significant influence on the number of thermally generated radicals [43]. Decomposing potato starch particles, involves taking treated potato starch granules, subjecting starch granules suspension to high pressure up to 1000 MPa followed by performing hydrolysis, separating, compacting and drying product has been developed [44]. Pressurization of the potato starch was performed in a standard hydraulic press cylinder piston device shown in Scheme 3.

Pressure changes will also affect enzyme catalysed reactions. Any reaction involving dissolved gases, e.g. oxygenases and decarboxylases, will be particularly affected by the increased gas solubility at high pressures. Caused by pressure the equilibrium position of the reaction will also be shifted due to any difference in molar volumes between the reactants and products. Pressure may lead to a doubling of the constant enzymatic rate k_{cat}, and/or a halving in the Michaelis constant K_m for a 1000 fold.

A kinetic characterization of pressure- and/ or temperature-induced enzyme inactivation has been published by Ludikhuyze, *et al.* [45] for the model enzyme system *Bacillus subtilis* -amylase. From isobaric and/or isothermal inactivation experiments, first-order inactivation rate constants were determined for about 50 combinations of pressure and temperature (0.1±750 MPa; 25±82C) and a pressure± temperature kinetic diagram was constructed.

The activity of partially purified blueberry peroxidase at different concentrations of hydrogen peroxide and phenlylenediamine as substrates and the effects of thermal and high pressure processing on the activity of the enzyme has been studied [46]. The thermal and high pressure inactivation kinetics of polyphenol oxidase (PPO) and

peroxidase (POD) in strawberry puree has been also studied [47]. The activity of these enzymes was investigated under different pressure and temperature conditions.

Pressurization/depressurization treatments caused a significant loss of strawberry polyphenoloxidase (PPO) (60%) up to 250 MPa and peroxidase (POD) activity (25%) up to 230 MPa, while some activation was observed for treatments carried out in 250-400 MPa range for both enzymes. Optimal inactivation of POD was using at 230 MPa and 437C in strawberry puree. High pressure and temperature effectively reduced POD activity in orange juice (50%). The effects of high pressure and temperature on pectin methylesterase PME activity in orange juice were very similar to those for POD [48].

The combined high pressure/thermal (HP/T) inactivation of tomato pectin methyl esterase (PME) and polygalacturonase (PG) was investigated [49]. The temperature and pressure ranges tested were from 60 °C to 105 °C, and from 0.1 to 800 MPa, respectively. Selective inactivation of either PME or PG was achieved by choosing proper combinations of *P* and *T*. The inactivation kinetics of these enzymes was measured and described mathematically over the investigated portion of the *P*/*T* plane. PME was found less sensitive to both heat and pressure when pH was raised above its physiological value but PG became more labile at higher pH values.

Polyphenoloxidases extracted from mushrooms and potatoes responded differently to pressure. The activity of a polyphenoloxidase extracted from mushrooms was found to decrease steadily with increasing applied pressure (100–800 MPa) and time (1–20 min) in phosphate buffer at pH 6.5. Complete inactivation was only achieved on treatment at 800 MPa for at least 5 min. The enzyme in potatoes steadily lost activity with increasing applied pressure although after 10 min at 800 MPa about 40% of the activity remained. The mushroom extract exhibited a marked increase in activity after treatment at 400 MPa for 10 min (about 140% of the value of the untreated sample) and even after 10 min at 800 MPa considerable activity remained. Possible reasons for these differences were discusssed [50].

5. Discussion

In this review we did a general overview of applications of high hydrostatic pressure techniques in the biological sciences: biochemistry, biology and biomedicine. We hope that this review help understand the importance of the high pressure in biological science. The high pressure shall apply to almost all areas of science and technology. Pressure is important in biochemistry – it has an impact on the structure of proteins and DNA. The pressure has a significant impact on growth and survival on the mid-water and bentonithic organisms. Pressure has also a practical use in the different branches of technology. Especially important is the use of pressure in foods technology. Applications of pressure are broad.

The question arises: why so few researches carried out under pressure? This is due to some problems with work under pressure. Obtain an adequate pressure is not easy. To work with the pressure needed are appropriate materials and equipment. Material from which devices are built to study under pressure must be hard and resistant to pressure. Working with pressures requires careful attention.

We believe that this review article drew readers to the importance of pressure in biological sciences, such as biochemistry, biology, food technology, or medicine.

6. Conclusions

The above data shows that the pressure is important in biology, biomedicine, food technology and other life sciences. It seems that the pressure has a greater importance in the life sciences than in physics and chemistry despite greater use of pressure in physical sciences.

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