Z Lebensm Unters Forsch (1995) 200: 3–13

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Review

Pasteurization of food by hydrostatic high pressure: chemical aspects

Bernhard Tauscher

Institute of Chemistry and Biology, Federal Research Centre for Nutrition, D-76131 Karlsruhe, Germany

Received October 4, 1994

Pasteurisierung von Lebensmitteln mit hydrostatischem Hochdruck: Chemische Aspekte

Zusammenfassung. Mit Hilfe hydrostatischen Hochdrucks pasteurisierte Lebensmittel werden in Japan bereits vermarktet. Auch in Europa und USA ist das Interesse an dieser Methode groß. Temperatur und Druck sind die wichtigsten Parameter, die den Zustand der Materie – auch von Lebensmitteln – beeinflussen. Während die Einwirkung von Temperatur auf Lebensmittel seit langem Gegenstand von Untersuchungen ist, ist der Einfluß des Druckes auch in Kombination mit Temperatur auf Lebensmittel ein relativ junges Forschungsgebiet. Generell sollten Vorgänge und Reaktionen in Lebensmitteln untersucht werden, die dem Prinzip von Le Chatelier unterliegen. Hierzu gehören chemische Reaktionen niedermolekularer und makromolekularer Verbindungen. Es werden theoretische Grundlagen und Beispiele für durch Druck beeinflußte Reaktionen vorgestellt.

Abstract. Food pasteurized by hydrostatic high pressure have already been marketed in Japan. There is great interest in this method also in Europe and USA. Temperature and pressure are the essential parameters influencing the state of substances including foods. While the influence of temperature on food has been extensively investigated, effects of pressure, also in combination with temperature, are attracting increasing scientific attention now. Processes and reactions in food governed by Le Chatelier's principle are of special interest; they include chemical reactions of both low- and macromolecular compounds. Theoretical fundamentals and examples of pressure affected reactions are presented.

Introduction

Hite and Bridgman were the first to report on the application of hydrostatic high pressure to biological material. It was in 1899 when Hite et al. exposed several foods and beverages to high pressure of up to about 680 MPa [1]; in 1914 Bridgman coagulated egg white under pressure [2]. It took a while for the next remarkable contributions to the influence of pressure on biological material including food to appear in the fifties. Pressure induced changes in biopolymers include also changes in spectral characteristics. The rapid development of spectroscopic methods opened up new fields of research. Today nearly all common biophysical methods are used also for pressure treated biological materials [3, 4]. After first spectroscopic studies on protein denaturation by high pressure in the seventies [5] essential papers were published among others by Morild [6], Heremans [7], Weber [8], Balny [4, 9], Kunugi [10], and Jaenicke [11].

Technical-scientific progress has led to a renaissance of food pasteurization by hydrostatic high pressure recently [12–15]. In the Japanese market high-pressure pasteurized food has been available for some years [16–19]. Food treated in this way keeps its original freshness, colour, flavour and taste. Other advantages of high-pressure treatment are the gradient-free immediate expansion of the pressure which is thus equally effective everywhere in the food. Pressure pasteurization is feasible also at room temperature and energy saving as compared to heat treatment. Other influences to be expected besides destruction of microorganisms (overviews in e.g. [20–23]) are: protein denaturation or modification, enzyme activation or inactivation, changes in enzyme substrate specifity, changes in the properties of polymer carbohydrates and fats. The revived interest in high-pressure pasteurization of food has raised many questions, among them those of the pressure-temperature behaviour of macromolecular food components such as proteins, lipids and polysaccharides; the mechanism of protein gelation and of the sol/gel behaviour of polysaccharides, for example, are not well understood.

Little is known so far about reactions of low-molecular compounds in the matrix 'food', i.e. in usually aqueous media. High pressure, on the other hand, has for long been a means of manipulating organic-chemical reactions (over-

views e.g. in [24–29]). High pressure influences organic reactions in general. So the question arises whether at pressure >500 MPa which is employed for food sterilization the chemical reactions supposed to occur are desirable or not.

Generally any process and any reaction in food are of interest to which the principle of Le Chatelier applies, according to which, under equilibrium conditions, a process associated with a decrease in volume is favoured by pressure, and vice versa.

The "driving force" of a reaction is the (negative) free enthalpy change $\Delta G = G^{(product)} - G^{(educt)}$ which depends upon the concentration of the initial material and of the products, but also on the chemism of the reaction, temperature and pressure. With ΔG being negative, the reaction is spontaneous, with ΔG being positive, it is not. Gibbs' fundamental equation (eq. 1) correlates pressure, temperature and chemical potential

$$dG = -SdT + VdP + \sum \mu_i dn_i \tag{1}$$

with S = entropy, V = volume, μ_i = chemical potential, and n_i = number of moles of i. Equation 2 correlates the pressure influence on G with the volume:

$$V = \left(\frac{\partial G}{\partial P}\right)_{T,n_i} \tag{2}$$

An increase of pressure has been found to change the reaction rate of chemical reactions in solution. This effect is low as compared to the influence of temperature: 100 MPa may be required to achieve the same effect as a temperature increase by 10 °C. While reactions when regarded as a function of temperature provide insight into the energy levels involved, functions of pressure shed light on the so-called "volume profiles" of the process. Pressure changes the physical properties – boiling point, melting point, density, viscosity, solubility, dielectric constant and conductivity – of the material under investigation. Melting points may rise with increasing pressure. Water is an exception as its volume increases during crystallization to ice modification I (Fig. 1). Pressure can hence be expected to bring about partial solidification also of foods. Lipids e.g. which are liquid under normal conditions crystallize under pressure. A pressure increase of about 100 MPa increases the viscosity of liquids by a factor of about 2; this influences diffusion controlled reactions significantly. Water is an exception again; its viscosity remains nearly constant. As the solubility of compounds [30] depends usually on the pressure, precipitations must be expected to occur in pressure treated food. Pressure influences the reaction rate and the chemical equilibrium of reactions in food.

Thermodynamic ($\Delta\overline{V}$) and kinetic ($\Delta V^{\ddagger}$) effects of pressure

Temperature and pressure are used to shift reaction equilibria. The shift is determined by the change in the chemical potentials of the components involved. The pressure dependence of the chemical potential μ_i of a dissolved compound i is shown in equation 3, where $\overline{V}_i$ = the partial molar volume of compound i.

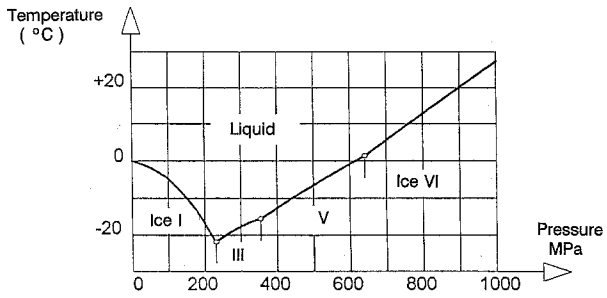


Fig. 1. Phase diagram of water

$$\left(\frac{\partial \mu_i}{\partial P}\right)_T = \overline{V}_i \tag{3}$$

In an ideal solution equation 4 correlates the chemical potential with the mole fraction (x); μ_i^o = the chemical potential of i in its standard state.

$$\mu_i = \mu_i^o + RT\mathrm{In}x_i \tag{4}$$

To a general chemical reaction at equilibrium (eq. 5)

$$aA + bB + \ldots \rightleftharpoons lL + mM + \ldots \tag{5}$$

equation 6 applies:

$$l\mu_L + m\mu_M + \ldots - a\mu_A - b\mu_B - \ldots = 0 \tag{6}$$

The equilibrium constant K_x as a function of pressure is shown in equation 7:

$$-RT\left(\frac{d\mathrm{In}K_X}{dP}\right) = l\overline{V}_L + m\overline{V}_M + \ldots$$
$$-(a\overline{V}_A + b\overline{V}_B + \ldots) = \Delta\overline{V} \tag{7}$$

where the equilibrium constant K_x is expressed in terms of mole fractions (eq. 8):

$$K_X = \frac{X_L^{\,l} X_M^{\,m} \cdots}{X_A^{\,a} X_B^{\,b} \cdots} \tag{8}$$

For the state of equilibrium of a chemical reaction under pressure the reaction volume $\Delta\overline{V}$ is decisive.

The influence of pressure on the reaction rate may be described by the transition state theory. The rate constant k_x of a reaction in a liquid phase (Eq. 9) is proportional to the quasi equilibrium constant $K_x^{\ddagger}$ for formation of the active complex of reactants.

$$aA + bB + \ldots \overset{K_x^{\ddagger}}{\rightleftharpoons} X^{\ddagger} \rightarrow products \tag{9}$$

In this way the pressure dependence of k_x is expressed in terms of the activation volume $\Delta V^{\ddagger}$ (eq. 10):

$$-RT\left(\frac{d\mathrm{In}k_X}{dP}\right)_T = \Delta V^{\ddagger} \tag{10}$$

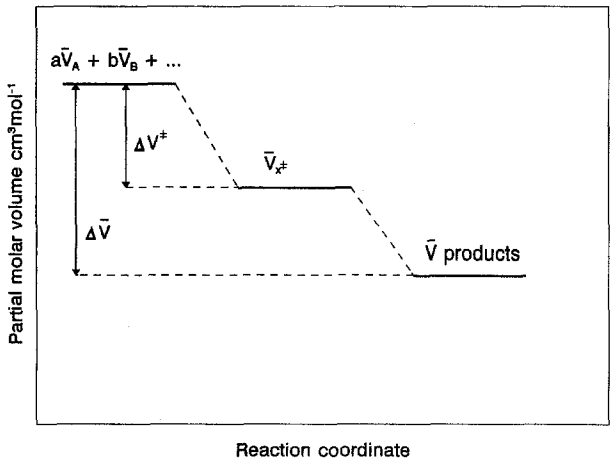


Fig. 2. Typical volume profile of reaction 9

Figure 2 shows the volume profile of reaction (9).

The partial molar volumes of dissolved components are influenced by 3 factors [31]: 1) by an intrinsic share of the dissolved component which is determined by a change in van der Waals radii; 2) by interactions of components and solvent, leading to electrostriction; and 3) by interaction of the component with all dissolved components including itself. Factor 3 is negligible in dilute solutions. Factor 1 (intrinsic factor) is assumed to be independent of solvent and concentration. By a first approximation the reaction volume corresponds to the sum of two volume parts, one of which is known as the intrinsic, the other as solvation volume (eq. 11).

$$\Delta\overline{V} = \Delta\overline{V}_{intr} + \Delta\overline{V}_{solv} \tag{11}$$

The intrinsic part ($\Delta\overline{V}_{intr}$) results from the motion of the atomic nuclei of the reacting component, and from changes in bonding length and angle during the formation of products. The solvation part ($\Delta\overline{V}_{solv}$) includes any change in volume associated with changes in polarity, electrostriction and dipole interaction with the solvent during the reaction. $\Delta V^{\ddagger}$ may be described in a similar manner (eq. 12):

$$\Delta V^{\ddagger} = \Delta V^{\ddagger}_{intr} + \Delta V^{\ddagger}_{solv} \tag{12}$$

In reactions without substantial contributions by solvation $\Delta V^{\ddagger}$ is determined by $\Delta V^{\ddagger}_{intr}$. In this case forming of a covalent bond between two atoms is assumed to be associated with a negative intrinsic contribution $\Delta V^{\ddagger}_{intr}$, while bond scission is characterized by a positive change in volume $\Delta V^{\ddagger}_{intr}$. Accordingly a bond-forming process is accelerated by pressure, while a bond-breaking one is delayed. When, however, charges appear in a transitional state or in the products formed, the situation is more complex because of interactions with the solvate shell. In reactions with strong changes in polarity $\Delta V^{\ddagger}_{solv}$ may be larger than $\Delta V^{\ddagger}_{intr}$. Polar reactions of this kind have been found to depend strongly on the polarity of the solvent.

It may be summarized that bond formation, charge separation and concentration of equal charges, and steric crowding result in volume contraction; bond scission,

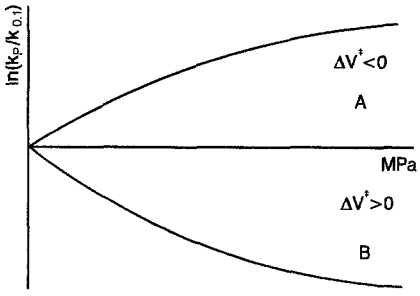


Fig. 3. Typical, non linear plots of in ($k_p/k_{0.1}$) vs pressure. (A) Cycloaddition reaction, (B) homolytic scission reaction

charge neutralization and delocalization result in volume expansion. An overview paper [32] of the activation and reaction volumes of some thousand organic and anorganic reactions may be helpful as a source of information about reactions among food components which are accelerated or inhibited by pressure.

When the density of a solution of known concentration and of the solvent are known, the apparent molar volume of the dissolved substance can be calculated. The partial molar volume is derived from the concentration dependence of the calculated values. Density measurements using modern equipment allow the partial molar volume to be calculated very precisely [33]. Hence the reaction volume $\Delta\overline{V}$ can be determined provided the density of all components involved is known. For rapid reactions special dilatometers are available allowing to measure $\Delta\overline{V}$ directly [34].

When reaction or activation volumes are derived from measurement of the equilibrium constant K or the rate constant k, one has to bear in mind that lnK or lnk are linear functions of pressure in the ideal case only; usually the function is non-linear. Therefore it is necessary to apply a standard pressure – usually 0 MPa – to compare reaction and activation volumes. O MPa is not significantly different to 0.1 MPa, i.e. atmospheric pressure. Equilibrium constants, furthermore, have to be expressed in terms independent of pressure as molefractions or molalities [35]. Usually volumes are derived from the slope of the curves shown in Fig. 3 at P = 0 MPa; they have been termed $\Delta\overline{V}_0$ and $\Delta V^{\ddagger}_0$, resp.

Reaction volumes of chemical reactions

Ionization of water, acids, phenols and amines

Water is one of the most important food ingredients especially of fruit and vegetable products. Therefore some characteristics of water under pressure will be presented. Compared to gas, water is nearly incompressible (by 4 Vol% at 100 MPa and 22 °C, and by 15 Vol% at 600 MPa and 22 °C). Adiabatic compression of water increases the temperature by about 3 °C per 100 MPa [36]. Phase transitions of water depend on pressure. At −22 °C and 210 MPa e.g. water is still liquid. That water exposed to pressure does not freeze at 0 °C is due to the fact that transition of the liquid to the ice I phase (Fig. 1) is accompanied by an increase in volume which counteracts

pressure [37]. At pressure above 210 MPa the melting point of ice rises with pressure, i.e. at 20 °C and 880 MPA, or 30 °C and 1036 MPa water solidifies. These effects may be used to thaw food quickly; the heat of transformation has to be added. Conceivable – but expensive – is also storage of non-frozen food under pressure at temperatures below zero.

Self-ionization of water is promoted by pressure. The ionization volume of water in H_3O^+ and OH^- ions is -22.2 cm³mol^{-1} at 25 °C. Volume contraction is brought about by strong electrostriction around the ions formed.

There is no example of ion formation from neutral molecules that is not associated with volume contraction. In large ions and in ions characterized by charge delocalization contraction is lower. This may be exemplified by the ionization volumes of phosphoric acid (eq. 13) at 25 °C [38].

$$H_3PO_4 \overset{-H^+}{\rightarrow} H_2PO_4^- \overset{-H^+}{\rightarrow} HPO_4^{2-} \overset{-H^+}{\rightarrow} PO_4^{3-} \quad (13)$$

$\Delta\overline{V}_1 = -16.3$ $\Delta\overline{V}_2 = -25.9$ $\Delta\overline{V}_3 = -36.0$ cm³mol^{-1} (25 °C)

This implies that pressure promotes ionization of phosphoric acid up to the phosphate ion. An example as interesting is the dissociation of diphosphoric acid (pyrophosphoric acid) under pressure at 25 °C (eq. 14) [39].

$$^-HO_3POPO_3H_2 \overset{-H^+}{\rightarrow} {}^-HO_3POPO_3H^- \overset{-H^+}{\rightarrow}$$
$$^-HO_3POPO_3^{2-} \overset{-H^+}{\rightarrow} {}^{2-}O_3POPO_3^{2-} \quad (14)$$

$\Delta\overline{V}_1 = -16.0$ $\Delta\overline{V}_2 = -20.7$ $\Delta\overline{V}_3 = -28.9$ cm³mol^{-1} (25 °C)

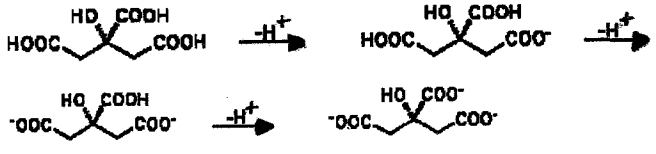
Carbonic acid responds to pressure by increased ionization suggesting also covalent bond formation between water and CO_2 [40] (eq. 15).

$$CO_2 + 2H_2O \rightarrow HCO_3^- + H_3O^+ \; HCO_3^- \overset{-H^+}{\rightarrow} CO_3^{2-} \quad (15)$$

$\Delta\overline{V}_1 = -26.0$ cm³mol^{-1} $\Delta\overline{V}_2 = -29.2$ cm³mol^{-1} (25 °C)

The dissociation of boric acid shows a reaction volume of -35.5 cm³ mol^{-1} at 25 °C. The dissociation behaviour of anorganic and organic acids under pressure has considerable influence on the selection of suitable buffer systems for e.g. protein studies.

Monobasic carboxylic acids approximate an ionization volume of about -14 cm³ mol^{-1} with increasing carbon number. The first and second ionization volumes of non-branched dibasic carboxylic acids [41] are close (-13 to -14 cm³ mol^{-1}), while those of oxalic acid are relatively distant to each other (-6.7 to -11.9 cm³ mol^{-1}). The ionization volumes of citric acid (eq. 16) increase as expected with advancing dissociation [38].



(16)

$\Delta\overline{V}_1 = -10.7$ $\Delta\overline{V}_2 = -12.3$ $\Delta\overline{V}_3 = -22.3$ cm³mol^{-1} (25 °C)

Phenols have larger negative ionization volumes than carboxylic acids. The value for phenol itself is comparable to that of water. Substituents at the aromatic ring influence the ionization behaviour of the phenolic OH group.

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (17)$$

$\Delta\overline{V} = -28.8$ cm³ mol^{-1} (25 °C)

The dissociation volume of ammonia (eq. 17) is higher than that of water [42]. The protonation of amines is promoted by pressure, as is the transition of NH_3^+-glycine and NH_3^+-alanine to the zwitterionic form (-7 to -8 cm³ mol^{-1}) [43]. However, proton transfer from $-NH_3^+$ to water is associated with a positive reaction volume.

Hydrogen bond formation

Formation of hydrogen bridges is supposed to be associated with a small volume contraction, due to the small diameter of the covalently bound hydrogen atom and the considerable length of the hydrogen-bridge bond. This has indeed been verified and explained for phenol as H donor [44]. The changes in volume are by few cm³ mol^{-1}. For the dimerization of carboxylic acids a stronger volume contraction at room temperature is expected because of the double H bridge bond. Dimerization of carboxylic acids in aqueous medium at 30 °C yielded larger reaction volumes indeed [45]. With increasing pressure above 200–300 MPa association is suppressed with increasing chain length. This may be due to hydrophobic interactions (see following section) of the alkyl groups in the dimer. In keto-enol tautomers pressure has been found to favour the keto form slightly, i.e. the cyclic structure of the enol form has a somewhat larger volume than the keto form, as has been shown in ethyl acetoacetate [46]. These examples demonstrate that formation of hydrogen-bridge bonds is not always associated with molar volume contraction due to a slight reduction of bond lengths. Volume contraction may be compensated or even overcompensated for by hydrophobic interactions or more voluminous cyclic structures.

Hydrophobic effects [47, 48]

Unpolar compounds tend to aggregate in aqueous media. Water molecules prefer binding to each other to binding to an unpolar hydrocarbon. Hydrophobic interactions are responsible for the stability of, and interactions among biological macromolecules as proteins and lipids, for the binding of substrates to enzymes, and for micelle formation. When taking place in aqueous solution, also simple organic reactions may exhibit hydrophobic effects. The hydrophobic effect increases in the presence of compounds such as lithium chloride which reduces the solubility of hydrocarbons in water. Lithium chloride could also be called "salting-out-agent". Some salts, among them guanidinium chloride e.g., enhance the solubility of hydrocarbons in water; they could accordingly be called "salting-in-agents". The intermolecular effects are not fully understood

[49]. Hydrophobic effects as a function of ions added deserve attention in all cases of pressure treatment.

Formation of covalent bonds

Covalent bonds are shorter than those involved in complex formation. Hydration of carbonyl compounds under high pressure is accompanied by volume contraction of about 10 cm^3 mol^{-1} [50]. When two covalent bonds are formed as e.g. in Diels-Alder reactions, larger volume contractions of 30–40 cm^3 mol^{-1} have been observed. The influence of pressure on this type of reaction has been studied extensively. In many Diels-Alder reactions activation and reaction volumes are of comparable size. This means similar volume requirements of product and transitional state.

Reaction and activation volumes of organic reactions may provide clues to potential reactions of food ingredients under high pressure. Of interest could be 1) the Menschutkin reaction, 2) reactions at C = C and C = 0 bonds, 3) [2+2] polar cycloadditions, 4) Diels-Alder reactions, and 5) solvolyses.

Activation volumes of chemical reactions

Homolytic and ionic reactions

Homolytic cleavage is expected to be accompanied by volume expansion: generally the activation volume of simple radical degradation processes is nearly +10 cm^3 mol^{-1} [32]. Processes of this kind are hence retarded by pressure. This applies also to other reactions in which neutral fragments form neutral molecules. Vice versa, bond formation among neutral particles is characterized by volume contraction. The propagation step during polymerization is greatly accelerated by pressure, and so is the hydrogen abstraction from a molecule by free radicals in a bimolecular process [29]. Termination steps during polymerization are retarded by pressure; this appears surprising at a first glance, as dimerization of radicals is involved. However, these processes are controlled by diffusion, and as viscosity increases with increasing pressure, diffusion is retarded.

In food, autoxidation e.g. of fat is important. Accordingly the initial reaction, i.e. formation of radicals from neutral molecules such as unsaturated fatty acids should be retarded by pressure, while propagation steps should be favoured. The effect of pressure on oxidative processes in foods has been little investigated. Oxidation of sardine lipids has been shown to be accelerated by pressure as a function of the duration of pressure treatment and pressure height [51]. The radical chemistry of polyunsaturated fatty acids (PUFA) under normal conditions has been investigated by several authors [52]. Autoxidation of linolenic acid under high pressure [53, 54] is complex: initially (350 MPa) more primary oxidation products form than under atmospheric pressure (Fig. 4), recognizable by formation of conjugated dienes with an absorption maximum at λ = 234 nm. At higher pressure the initial change is less distinct. Table 1 shows the decrease in linolenic acid as a function of pressure time and pressure height. Accordingly, high pressure (600 MPa) has a greater protective effect on the autoxidation of linolenic acid than medium pressure. Reaction products of subsequent reactions remain to be fully explored. Investigations are necessary also on the storability of high-pressure treated products which are rich in polyunsaturated fatty acids.

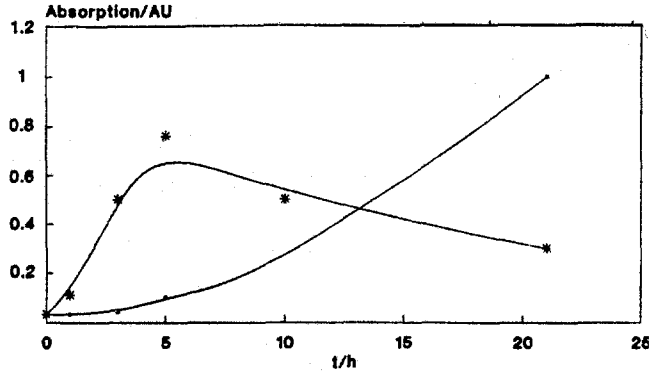


Fig. 4. Conjugated diene absorption (234 nm) after pressure treatment of linolenic acid. -⊡- 0.1 MPa; -✳- 350 MPa

Table 1. Linolenic acid content after different times of pressure treatment [54]

	0,1 MPa	350 MPa	600 MPa
1 h	99%	80%	91%
3 h	76%	73%	89%
21 h	52%	72%	80%

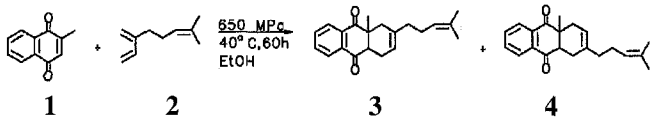
Diels-Alder and Menschutkin reactions

Diels-Alder reactions as [2 + 4] cycloadditions have been studied extensively under pressure. The pressure induced acceleration in this reaction is one of the largest. The reaction between electron-rich dienes and electron-deficient dienophiles is characterized by large negative activation volumes. In some cases activation volumes exceed reaction volumes. The activated complex hence has a very tight structure. The same applies also to inverse Diels-Alder reactions.

The selectivity of a reaction can be controlled by pressure; in this way the product whose reaction volume is more negative is favoured. Optimal effects have been obtained by simultaneous variation of pressure and temperature [55]. The chemo-and endo-selectivity of Diels-Alder reactions in aqueous media is strongly affected by hydrophobic interaction [56, 57].

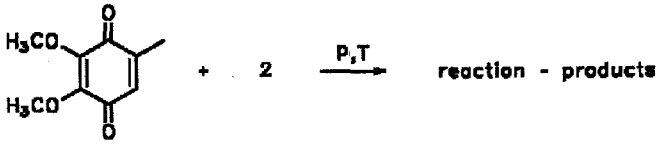
In food, Diels-Alder products may form during thermal treatment. Unsaturated fatty acids may turn into conjugated fatty acids which may react to Diels-Alder adducts [58]. Parsley-, lavender- and tagetes oil may also contain Diels-Alder products involving the influence of terpenoidal compounds [59, 60]. Retinol may form dimer products as well [61].

Quinones may act as dienophiles, and conjugated terpenoids as dienes [62]. Vitamin K_3 (1) reacts with myrcene (2) to form the Diels-Alder products 3 and 4 at a ratio of about 1:1 (eq. 18).



1 2 3 4 (18)

Vitamin K_1 and K_2 have been found to react much less with myrcene under these conditions. Coenzyme Q_0 (5) also reacts with 2 while the homologous ubiquinones fail to react (eq. 19).



5 (19)

It remains to be investigated whether detectable levels of Diels-Alder products result from high-pressure treatment of food.

Cycloadditions governed by dipolar or concerted mechanisms are accompanied by strong volume contraction in the transition state and accelerated by pressure [63]. Besides [2+4] also polar [2+2] cycloadditions are of interest. Pressure may affect the ratio between [2+2] and [2+4] cycloadducts in reactions involving both; the reaction showing the largest negative activation volume will dominate. Cycloadditions involving a dipol intermediate have been found to show an activation volume as large as that of Diels-Alder reactions. Nothing has been known so far about reaction products of this kind in food.

Large negative activation volumes are characteristic of reactions involving charges arising in the product, e.g. quarternization of nitrogen, formation of sulphonium- and phosphonium salts. These reactions have been known as Menschutkin reaction (eq. 20).

$$R_3N + R' - X \rightarrow R_3N^+ - R' + X^- \qquad (20)$$

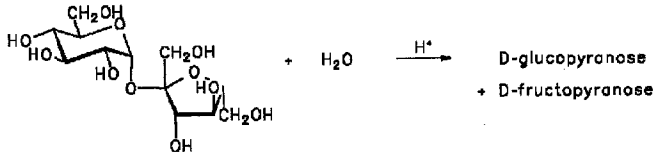
$\Delta\overline{V}^{\ddagger}$ up to -50 cm^{3}mol^{-1}

Also dichloro- and dibromomethanes may be used as alkylating agents under pressure [64]. Whether Menschutkin products form in pressure treated foods remains to be investigated.

Solvolytic reactions

Other organic reactions and reaction types favoured by pressure include e.g. nucleophile substitution reactions or additions at double bonds [65, 66]. The significance of these in food is difficult to estimate. Of interest are also solvolytic reactions, especially those catalyzed by acids where the solvent acts as nucleophile (Lewis base). Solvolytic reactions of neutral substrates are accelerated by increasing pressure. In S_N1 reactions increase of the $V^{\ddagger}_{intr}$ is overcompensated by contraction of the solvent in the transition state. In S_N2 reactions volume contraction is brought about by formation of a covalent bond and by electrostriction in the activation step. Activation volumes are usually between -10 and -35 cm^3 mol^{-1}.

Acid catalyzed hydrolyses of ethers, esters, acetals, and ketals are governed by A-1 or A-2 mechanisms. A-2 mechanisms are accelerated by pressure, while A-1 ones are retarded. Most of the ketals and acetals are assumed to be controlled by the A-1 type of process. Ethers and simple esters solvolyze quicker (A-2), orthoesters slower under pressure. The retardation is due to the substrates' capability of forming relatively stable carbenium ions. A classical example of A-1 hydrolysis pertains to the inversion of sucrose (eq. 21) [67].



(21)

$\Delta\overline{V}^{\ddagger} = +6.0$ cm^{3}mol^{-1} (25 °C)

Hydrolysis of simple glucosides of monosaccharides seems to depend on the form – furanose or pyranose – of their carbohydrates. Hydrolysis of the glucopyranose form is retarded by pressure, while the glucofuranose form usually shows low negative activation volumes. This would mean that the first follows an A-1 mechanism, the latter an A-2 one [68]. Low volume contractions measured in model systems are supposed to be of no importance for corresponding reactions in pressure treated food. Neither is mutarotation assumed to be significantly influenced by pressure. Activation volumes of the transformation of α-glucopyranose to β-glucopyranose, and vice versa (eq. 22) are of the same order of magnitude.

$$\alpha\text{–glucopyranose} \underset{k_\beta}{\overset{k_\alpha}{\rightleftharpoons}} \beta\text{–glucopyranose} \qquad (22)$$

$\Delta V^{\ddagger}_a = -11.7$ $\Delta V^{\ddagger}_b = -10.8$ cm^{3}mol^{-1} (25 °C)

The reaction-rate constant $k = k\alpha + k\beta$ increases with increasing pressure, while the equilibrium constant $K_{eq} = k\alpha/k\beta$ is independent of pressure. Due to the negative activation volumes, however, equilibrium is achieved faster under pressure [69].

Effects of pressure on biopolymers

Many studies on the influence of high pressure on proteins and enzymes were related to studies on the mechanisms of adaptation of life to-deep sea conditions [70–72]. It is interesting that adaptation to low temperatures had probably priority to adaptation to high pressure. The fall in temperature in the transition to deep-sea conditions (Mariana trench) delays chemical reactions by 400%, while an increase in pressure causes a change by 15% only [73].

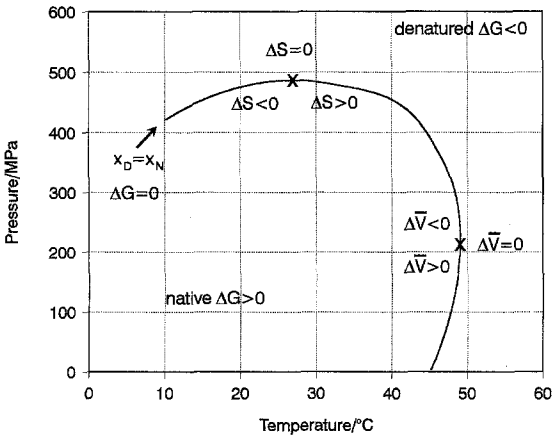


Fig 5. Pressure/temperature phase diagram of proteins, according to [74]. x = molar fraction denatured/native

Changes in proteins/enzymes at pressure of >120 MPa have been investigated extensively.

The structure of proteins changes under the influence of pressure. Pressure favours the dissociation of oligomeric proteins or of complex macromolecular systems, as well as unfolding of protein chains. A protein in its native state is stabilized by

- covalent bonds including disulfide bridges
- electrostatic interactions (ion pairs, polar groups)
- hydrogen bridges
- hydrophobic interactions.

Pressure affects

- the quarternary structure (e.g. through hydrophobic interactions)
- the tertiary structure (e.g. through reversible unfolding)
- the secondary structure (irreversible unfolding).

Denaturation of single-chain proteins may be regarded as 2-component system (native $\rightleftharpoons$ denaturated); its pressure dependence is shown in Fig. 5 and has been exemplified by chymotrypsinogen [74]. This pressure/temperature dependence was first demonstrated by Suzuki in 1960 [75]. Differences in temperature and pressure denaturated proteins and gels are of interest for food industry. As is shown in Fig. 5, the denaturation temperature rises initially with increasing pressure. At maximal transition temperature the sign of $\Delta\bar{V}$ changes; from this point on the protein denaturates at lower temperatures at the given pressure. At maximal transition pressure the sign of ΔS changes; from this point on the protein denaturates at lower pressure at the given temperature. It is hence possible to denaturate proteins by pressure at low temperatures. This aspect deserves particular attention; proteins e.g. in meat and fish may be denaturated at sufficiently low temperatures and low pressure; the products thus obtained may show special textural properties. One should remember that water is still liquid at –20 °C when exposed to pressure of 200 MPa.

Native proteins are stable in a narrow range only; the difference in energy between native and denaturated state is small. For small proteins transition to the denaturated state is a cooperative process without detectable intermediates along the reaction pathway, while for oligomeric proteins or proteins with several domains denaturation is a complex process involving many intermediates. The determination of kinetic and thermodynamic parameters of the transition native – denaturated provides information about internal and external factors effecting stability. Also renaturation processes and their physico-chemical parameters are sources of information about folding processes leading e.g. to active enzymes.

Generally most of the proteins denaturate when exposed to pressure above 400 MPa. Measurements of changes in volume have shown that β-sheet structures are more stable against pressure than α-helical ones. β-sheet structures are nearly incompressible, α-helical structures form quicker than β-sheet structures. Compact proteins of low flexibility show little compressibility and high stability. The mechanisms of protein denaturation by hydrostatic pressure and the compressibility of proteins have been subject of many reviews, e.g. [6, 76, 77].

Oligomeric proteins dissociate to subunits, with $\Delta\bar{V}$ being negative. After dissociation subunits may aggregate or denaturate. At pressure above 200 MPa chains start unfolding and subunits of dissociated oligomers start reassociating. β-Casein e.g. depolymerizes reversibly below 150 MPa, at higher pressures a temperature-dependent reversible reassociation is observed. Processes of this kind are frequently accompanied by hysteresis [76]. Examples of protein dissociation are shown in Table 2.

Enzymes are proteins of biocatalytic function which accelerate chemical metabolic reactions and which, especially in the food sector, are gaining in importance with regard to selective substance transformations. Pressure affects not only the proteins, but also the reaction rate and equilibrium state in accordance with the fundamental laws of thermodynamics and kinetics.

In enzyme reactions, two steps have to be considered: 1) substrate binding, resulting in a complex of substrate and enzyme, and 2) the catalytic step, activating the enzyme-substrate complex. Both steps may be subdivided into subprocesses [6, 82] in which hydrophobic or polar interactions, rearrangement of water molecules, and changes in conformation are involved. The change in volume mea-

Table 2. Typical volume changes of protein dissociation/association processes

Protein	Reaction	$\Delta\bar{V}$ (cm^3 mol^{-1})	Ref
F-actin	Dissociation, up to 240 MPa reversible	–74 up to –328	78
Lactatede-hydrogenase	Dissociation ($M_4 \rightarrow 4M$) holoenzyme, satd. with NADH	–500 –390	11
7S storage globulin (pea seeds)	Dissociation	–146	79
Ribosome	Dissociation, E. coli 70S→50S+30S	<–240	11
Tryptophane-synthase	Dissociation β_2-subunit	–170	80
Arc repressor	Dissociation	–100	81

Table 3. Typical behaviour of enzymes under high hydrostatic pressure

Enzyme	Condition	Reaction	Ref.
Pectin methylesterase	600 MPa, 20' 20 °C, pH 2,5–4,5	No complete inactivation	83
Polyphenoloxidase	500 MPa, 10' 25 °C, pH 6,5	Activation (pears and onions) followed by Inactivation	84 85 86
Lipoxygenase	600 MPa, 30' 40 °C, pH 7,8	Inactivation	87
Taka-amylase A	400–700 MPa 25–45 °C pH 6,8	Inactivation	88
Actomyosin Mg-ATPase	700 MPa 30–60' 0 °C, pH 7,0	No complete inactivation	89

sured is the sum of all individual changes in volume. Formation of ligand-protein complexes may be accompanied by an increase in packing density. Substrate binding then increases the stability of the complexes due to reduced flexibility. In high substrate concentrations, the activation volume measured is governed by changes in volume during the catalytic step. In low substrate concentrations the activation volume may be concealed by a negative binding volume contribution. Therefore kinetic measurements of isolated enzymes have to be carefully checked for biological relevance with regard to ligand concentration. The influence of pressure on the reaction rate of enzymes may lead to different rates for different substrates. This change in substrate specifity under pressure has been observed e.g. in nucleases and hydrolases [10].

In recent years inactivation of microorganisms has been the primary goal of pressure treatment; now attention is shifting to food enzymes. Pressure may influence enzymes in many ways; it may e.g. increase enzyme activity, change the substrate specifity, and even inactivate enzymes. Table 3 shows influences of pressure on typical enzymes.

Sol-gel transition occurs in proteins and polysaccharides in solution. Gelatine and polysaccharides are regarded as systems showing the charactistics of common polymer solutions. Globular proteins, however, yield gels with properties resembling those of colloids and emulsions. It is of considerable importance to food technologists that temperature induced gels of ovalbumin, soy protein and carrageen are destabilized by pressure, while those of agarose and gelatine are stabilized. The mechanical properties of temperature and pressure induced gels of food proteins are different. Pressure induced gels are soft and glossy. Hardness increases with increasing pressure, while adhesiveness decreases.

Applications

Experiences from the use of high pressure have been reported for many foods. The following list does not claim completeness, but may be helpful to anyone concerned with high-pressure processing.

Hen's eggs

Formation of gels up to 700 MPa [90–92]; destabilization of heat induced gels up to 300 MPa [93]; removal of cholesterol by supercritical carbondioxid [94].

Milk and dairy products

Crystallization of milk fat [95]; influence on milk coagulation [93]; physico-chemical changes in skim milk up to 600 MPa [96, 97] and in acid induced gels of skim milk [98]; cold sterilization [99], stability of immunoglobulins (in the colostrum of cattle) during cold sterilization [100], inactivation of lactobacilli, E. coli [101] and listeria [102]; soft cheese [103], yoghurt [104], fast ripening of cheese [105].

Fish and fish products

Products of novel texture in which the aroma of the meat of sardines, mackerels, carp, thuna, pollack, bream and cuttlefish is largely retained [106–113] (see also review by Ohshima, [114]); sterilization of sea urchin eggs [115]; studies of lipidoxidation [51, 116]; texturization of water-soluble fish proteins [117].

Meat and meat products

Novel roastbeef by pretreatment at 250 MPa, and novel bacon by pressure treatment before smoking [118]; tenderizing of beef, pork, poultry- and rabbit meat [108, 118–124]; studies on gel formation [125, 126] and its influence on taste [127]; inactivation of germs in, and aspects of storage of minced meat [128, 129]; foie gras of excellent quality and extended shelf-life [130]; stability of thiamine in rehydrated pork [131].

Other polymeric food components and ingredients

Pressure induced sol-gel transitions of various starches [132–134] and alginates [135]; opposite sol-gel transition behaviour of carrageen and agarose under pressure [93, 136]; pectin extracted at 400 MPa from tangerines contains less galacturonic acid and methoxyl groups; the gel of pressure extracted pectin has different properties compared to gel of heat extracted pectin [137]; increased gel stability of gelatine up to 200 MPa [93].

Cereals and cereal products

Improved cooking characteristics and reduced allergenic potential [138]; different physical properties of pressure induced gels from soybeans [90, 91]; changed hydration of corn [139]; sensory properties of pressure treated rice [140]; cake, crackers and wine of rice [140–143]; decontamination of spaghetti and rice products [144].

Vegetables and vegetable products

Blanching of potato cubes [145]; preservation of pickles [146], tomato juice [147, 148] and onions [85]; influence on the hardness of radish [150]; inactivation of bean lectins [151]; decontamination of pepper and spice mixtures [152–154]; influence on paprika pigments [155].

Fruit and fruit products

Preparation of fruit products [156, 157]; sterilization of honey [158]; changes in the volatile compounds of peaches [159] and grapefruit [160]; changes in the composition of tea [161]; pasteurization of beverages [162] and of citrus juice in particular [163–165].

Cryomethods

Storage of pork and beef at 5 to −20 °C and 50–200 MPa [166]; pressure shift freezing of soybean noodles and tofu [167, 168]; thawing of thuna at 150 MPa [169]; use of pressure disinfected ice-nucleation active bacteria [170].

Prospect

High hydrostatic pressure has been used in the food sector to inactivate microorganisms. Protein denaturation, enzyme inactivation, protein gelation and the sol-gel behaviour of polysaccharides under pressure are contributions of unforeseeable importance for the food sector in particular. Chemical aspects of pressure treatment of food are manifold. Optimal treatment is required in any case. Products obtained in this way retain their original state of freshness but may have been changed in texture; so interesting innovative products will become available.

Acknowledgements. The author thanks his coworkers, especially Dr. Peter Butz, for their scientific and technical contributions to the project, and Sybill Saupe for the translation. Credit is due also to Prof. Dr. H. Ludwig, University of Heidelberg, for his critical comments and for helpful discussions. The project was supported by the Federal Ministry of Agriculture, Forestry and Food.

References

1. Hite BH (1899) West Virginia Agricultural Exp Station Bulletin 58:15
2. Bridgman PW (1914) J Biol Chem 19:511
3. Jonas J (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 123–127
4. Balny C, Masson P (1993) Food Rev Int 9:611
5. Zipp A, Kauzmann W (1973) Biochemistry 12:4217
6. Morild E (1981) Adv Protein Chem 34:93
7. Heremans K (1982) Annu Rev Biophys Bioeng 11:1
8. Weber G, Drickamer HG (1983) Q Rev Biophys 16:89
9. Balny C, Masson P, Travers F (1989) High Pressure Res 2:1
10. Kunugi S (1993) Prog Polym Sci 18:805
11. Gross M, Jaenicke R (1994) Eur J Biochem 221:617
12. Heremans K (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 37–44
13. Masson P (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 89–99
14. Hayashi R (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 244, J Libbey, London, pp 185–193
15. Cheftel JC (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 195–210
16. Gekko K, Fukamizu M (1991) Agric Biol Chem 55:2427
17. Hayashi R (1992) (ed) High Pressure Bioscience and Food Science. San-Ei Shuppan, Kyoto
18. Ogawa H, Fukuhisa K, Kubo Y, Fukumoto H (1990) Agric Biol Chem 54:1219
19. Hori K (1992) Japan Food Science 31:35
20. Butz P, Ludwig H (1986) Physica 139 and 140B:875
21. Hoover DG, Metrick C, Papineau AM, Farkas DF, Knorr D (1989) Food Technol 43:99
22. Ludwig H, Bieler C, Hallbauer K, Scigalla W (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM, J Libbey, London, pp 25–32
23. Knorr D (1993) Food Technol 47:156
24. Le Noble WJ, Kelm H (1980) Angew Chem 92:887
25. Isaacs NS (1981) (ed) Liquid phase high pressure chemistry. Wiley, Chichester New York Brisbane Toronto
26. Le Noble WJ (1988) (ed) Organic high pressure chemistry. Elsevier, Amsterdam Oxford New York Tokyo
27. Jurczak J, Baranowski B (1989) (eds) High pressure chemical synthesis. Elsevier, Amsterdam Oxford New York Tokyo
28. Matsumoto K, Acheson RM (1991) (eds) Organic synthesis at high pressures. Wiley, New York Chichester Brisbane Toronto Singapore
29. Winter R, Jonas J (1993) (eds) High pressure chemistry, biochemistry and material science. Kluwer, Dordrecht Boston London
30. Taniguchi Y, Senoo M, Hara K (1994) (eds) High pressure liquids and solutions. Elsevier, Amsterdam London New York Tokyo, pp 1–17
31. Hamann SD (1980) Rev Phys Chem Jpn 50:147
32. Van Eldik R, Asano T, Le Noble WJ (1989) Chem Rev 89:549
33. Le Noble WJ, Asano T (1975) J Org Chem 40:1179
34. Uemoto M, Hashitani T (1985) J Chem Soc Faraday Trans I 81:2333
35. Le Noble WJ (1987) In: Van Eldik R, Jonas J (eds) High pressure chemistry and biochemistry. Reidel, Dordrecht Boston Lancaster Tokyo, pp 279–293
36. Makita T, Hane H, Kanyama N, Shimizu K (1991) In: Hayashi R (ed) High pressure science for food. Ed San-Ei Pub Co, Kyoto, pp 93–100
37. Deuchi T, Hayashi R (1991) In: Hayashi R (ed) High pressure science for food. Ed San-Ei Pub Co, Kyoto, pp 101–110
38. Kitamura Y, Itoh T (1987) J Solution Chem 16:715
39. Surdo AL, Bernstrom K, Jonsson CA, Millero FJ (1979) J Phys Chem 83:1255
40. Barbero JA, Hepler LG, McCurdy KG, Tremaine PR (1983) Can J Chem 61:2509
41. Høiland H (1975) J Chem Soc Faraday Trans I 71:797
42. Read JA (1982) J Solution Chem 11:649
43. Lepori L, Mollica V (1980) Z Phys Chem NF 123:51
44. Sawamura S, Taniguchi Y, Suzuki K (1988) Ber Bunsenges Phys Chem 92:880
45. Suzuki K, Taniguchi Y, Watanabe T (1973) J Phys Chem 77:1918
46. Jouanne J, Heidberg J (1972) J Magn Reson 7:1
47. Ben-Naim (1980) (ed) Hydrophobic Interactions. Plenum Press, New York
48. Hvidt A (1983) Ann Rev Biophys Bioeng 12:1
49. Breslow R (1991) Acc Chem Res 24:159
50. Lewis CA, Wolfenden R (1973) J Am Chem Soc 95:6685

51. Tanaka M, Xueyi Z, Nagashima Y, Taguchi T (1991) Nippon Suisan Gakkaishi 57:957
52. Frankel NE (1991) J Sci Food Agric 54:495
53. Kowalski E, Tauscher B, Ludwig H (1994) In: Schmidt SC, Shaner JW, Samara GA, Ross M (eds) High-pressure science and technology 1993. AIP Conference, Proceedings 309, pp 1333–1336
54. Kowalski E: Thesis, Universität Heidelberg 1995
55. Klärner FG (1989) Chemie in unserer Zeit 23:53
56. Jenner G (1994) Tetrahedron Letters 35:1189
57. Li CJ (1993) Chem Rev 2023
58. Christopoulou CN, Perkins EG (1989) J Am Oil Chem Soc 66:1360; see also: Adelhardt R, Spiteller G (1993) Fat Sci Technol 95:85
59. Shaath NH, Griffin P, Dodeian S, Paloympis L (1986) In: Lawrence BM, Mookherjce BD, Willis BJ (eds) Flavors and fragrances: a world perspective. Proceedings of the 10th international Congress of Essent Oils, Fragrances and Flavors. Washington DC, USA 16–20 Nov, pp 715–729
60. Kaiser R, Lamparsky D (1984) Helv Chim Acta 67:1184
61. Burger BV, Garbers CF (1973) J Chem Soc Perkin Trans I 59
62. Ludwig H, Marx H, Tauscher B (1994) Eur High Pressure Research Group, 32th Annual Meeting, Brno, p 100
63. Scheeren JW (1989) In: Jurczak J, Baranowski B (eds) High pressure chemical synthesis. Elsevier, Amsterdam Oxford New York Tokyo, pp 168–209
64. Almarzoqi B, George AV, Isaacs NS (1986) Tetrahedron 42:601
65. Jenner G (1993) In: Jurczak J, Baranowski B (eds) High pressure chemical synthesis. Elsevier, Amsterdam Oxford New York Tokyo, pp 345–366
66. Hill JS, Isaacs NS (1986) Tetrahedron Lett 27:5007
67. Moriyoshi T (1971) Bull Chem Soc Jpn 44:2582
68. Isaacs NS, Javaid K, Capon B (1982) J Chem Soc Perkin II 101
69. O'Connor CJ, Odell AL, Bailey AAT (1983) Aust J Chem 36:275
70. Certes A (1884) Compt Rend 98:690
71. Sleigh MA, MacDonald AG (1972) (eds) The effects of pressure on organisms. Symp Soc Exp Biol 26
72. Jannasch HW (1987) In: Jannasch HW, Marquis RE, Zimmermann AM (eds) Current perspectives in high pressure biology. Academic Press, London, pp 1–15
73. Jaenicke R (1988) Forum Mikrobiologie 11:435
74. Hawley SA (1971) Biochemistry 10:2436
75. Suzuki K (1960) Rev Phys Chem of Japan 29:91
76. Weber G (1993) In: Van Eldik R, Jonas J (eds) High pressure chemistry and biochemistry, pp 471–487
77. Gekko K, Hasegawa Y (1986) Biochemistry 25:6563
78. Garcia CRS, Amaral JR JA, Abrahamsohn P, Verjorski-Almeida S (1992) Eur J Biochem 209:1005
79. Pedrosa C, Ferreira ST (1994) Biochemistry 33:4046
80. Silva JL, Miles EW, Weber G (1986) Biochemistry 25:5780
81. Silva JL, Silveira CF, Correia A, Pontes L (1992) J Mol Biol 223:545
82. Butz P, Greulich KO, Ludwig H (1988) Biochemistry 27:1556
83. Ogawa H, Fukuhisa K, Kube Y, Fukumoto H (1990) Agric Biol Chem 54:1219
84. Asaka M, Hayashi R (1991) Agric Biol Chem 55:2439
85. Butz P, Koller WD, Tauscher B, Wolf S (1994) Lebensm Wiss Technol 27:463
86. Knorr D (1993) Food Technol 47:150
87. Kowalski E, Ludwig H, Tauscher B (1994) 23rd Steenbock Symposium on High Pressure Effects in Molecular Biophysics and Enzymology, Madison, May 15–19, P-9
88. Makimoto S, Mori A, Taniguchi Y (1992) Zairyo 41:330
89. Ko WC, Tanaka M, Nagashima Y, Taguchi T, Amano K (1991) J Food Sci 56:338
90. Okamoto M, Kawamura Y, Hayashi R (1990) Agric Biol Chem 54:183
91. Goto H, Kajiya N, Noguchi A (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 321–329
92. Doi E, Shimizu A, Oe H, Kitabatake N (1991) Food Hydrocolloids 5:409
93. Gekko K (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 105–113
94. Budde A, Knorr D (1990) In: Spiess WEL, Schubert H (eds) Engeneering and Food. Elsevier, London, pp 159–165
95. Buchheim W, Prokopek D (1992) Dtsch Milchwirtsch 43:1374
96. Johnston DE, Austin BA, Murphy RJ (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 243–247
97. Shibauchi Y, Yamamoto H, Sagara Y (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 239–242
98. Johnston DE, Austin BA, Murphy RJ (1993) Milchwissenschaft 48:206
99. Cuoghi F (1993) Ind Aliment 32:956
100. Tonello C, Largeteau A, Jolibert F, Deschamps A, Demazeau G (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 249–254
101. Kobayashi S (1992) Project abstracts of the Japanese R&D Association for High Pressure Technology in Food Industry
102. Jewett FF jr, Hoover DG (1994) Institute of Food Technologists Annual Meeting, Atlanta June 25–29, Session 19–3
103. Kumeno K, Nakahama N, Honma K, Makino T, Watanabe M (1993) Biosci Biotech Biochem 57:750
104. Tanaka T, Hatanaka K (1992) Nippon Shokuhin Kogyo Gakkaishi 39:173
105. Yokoyama H, Sawamura N, Motobayashi N (1991) Eur Patent App N °91306976.1
106. Wada S (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 235–238
107. Shimada A, Kasai M, Yamamoto A, Hatae K (1990) Nippon Shokuhin Kogyo Gakkaishi 37:511
108. Yoshioka K, Kage Y, Omura H (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 325–327
109. Shoji T, Saeki H, Wakemeda A, Nakamura M, Nonaka M (1990) Nippon Suisan Gakkaishi 56:2069
110. Ko WC, Tanaka M, Nagashima Y, Taniguchi T, Amano K (1990) J Food Sci 56:338
111. Ishikawa M, Sakai K, Yamaguchi T, Rachi S (1991) In: Hayashi R (ed) High pressure science for food. San-Ei Publ Co, Kyoto, pp 309–316
112. Reference deleted
113. Nagashima Y, Ebina H, Tanaka M, Taniguchi T (1993) Food Research Int 26:119
114. Ohshima T, Ushio H, Koizumic C (1993) Trends Food Sci Technol 4:370
115. Yukizaki C, Kawano M, Tsumagari H (1993) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 228–235
116. Petillo D, Hultin HO (1994) Institute of Food Technologists Annual Meeting, Atlanta June 25–29, Session 71B–10
117. Okazaki E (1992) In: Hayashi R (ed) High Pressure Bioscience and Food Science. San-Ei Shuppan, Kyoto, pp 314–320
118. Nose M, Yamagishi S, Hattori M (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 321–323
119. Ohmori T, Shigehisa T, Taji S, Hayashi R (1991) Agric Biol Chem 55:357
120. Suzuki A, Watanabe M, Iwamura K, Ikeuchi Y, Saito M (1990) Agric Biol Chem 54:3085
121. Suzuki A, Suzuki N, Ikeuchi Y, Saito M (1991) Agric Biol Chem 55:2467
122. Kim K, Ikeuchi Y, Suzuki A (1992) Meat Science 32:237
123. Yoshioka K, Kage Y, Omura H (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 325–327

124. Deschamps O, Cottin P, Largeteau A, Demazeau G, Ducastaing A (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 175–177
125. Iso N, Mizuno H (1991) Shokuniku nikansuru Josei Kenkyu Chosa Seika Hokokusho 9:23
126. Iso N, Mizuno H, Ogawa H, Iso S (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 306–313
127. Suzuki A, Honma N, Fukuda A, Hirao K, Uryu T (1994) Meat Science 37:369
128. Carlez A, Cheftel JC, Rosec JP, Richard N, Saldana JL, Balny C (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 365–368
129. Carlez A, Rosec JP, Richard N, Cheftel JP (1994) Lebensm Wiss Technol 27:48
130. El Moueffak, Cruz C, Antoine M, Montury M, Demazeau G, Largeteau A, Le Ba D, Roy B, Zuber F (1993) Eur High Pressure Research Grp , 31th Annual Meeting, Belfast 30.8.–3.9.
131. Bognar A, Butz P, Kowalski E, Ludwig H, Tauscher B (1993) Bioavailability '93, Berichte der Bundesforschungsanstalt für Ernährung BFE-R–93–01:352
132. Ezaki S, Hayashi R (1992) In: Hayashi R (ed) High Pressure Bioscience and Food Science. San-Ei Shuppan, Kyoto, pp 115–121
133. Kudta E, Tomasik P (1992) Starch Stärke 45:19
134. Vainionpä J, Fossell P, Virtanen T (1993) Starch Stärke 45:19
135. Hirano R, Tobitani A, Shioya T (1992) In: Hayashi R (ed) High Pressure Bioscience and Food Science. San-Ei Shuppan, Kyoto, pp 200–203
136. Gekko K, Kasuya K (1985) Int J Biol Macromol 10:299
137. Kuribayashi T (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 337–339
138. Inoue T (1991) Jpn Patent App N °PCT/JP91/01805
139. Gunasekaran S, Farkas DF (1988) Trans ASAE 31:1589
140. Kuchiba N (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 104–108
141. Yamazaki A, Sasagawa A, Kinefuchi M, Yamada A (1993) 6. Symposium High pressure and bioscience, Kyoto August 19–21
142. Sasagawa A, Kinefuchi M, Yamazaki A, Yamada A (1993) 6. Symposium High pressure and bioscience, Kyoto August 19–21
143. Hara A, Nagahama G, Ohbayashi A, Hayashi R (1990) Nippon Nogeikagaku Kaishi 64:1025
144. Walker M, Farkas D (1994) Institute of Food Technologists Annual Meeting, Atlanta June 25–29, Session 79E-11
145. Esthaghi M, Knorr D (1993) J Food Sci 58:1371
146. Matsumoto T (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 349–354
147. Inakuma T, Sato T, Ishiguro Y (1994) Shokuhin Kogyo (Food Industry) 37:44
148. Ishiguro Y, Sanoda Y, Sato T, Okamoto T, Okamoto K, Sakamoto H, Inakuma T (1993) Nippon Nogei Kagakkaishi 67:1701
149. Butz P, Koller WD, Tauscher B, Wolf S (1994) Lebensm Wiss Technol 27: 463 and unpublished results
150. Kasai M, Hatae K, Shimada A (1993) 6.Symposium High pressure and bioscience, Kyoto August 19–21
151. Butz P, Fister H, Tauscher B (1994) Eur High Pressure Research Grp, 32th Annual Meeting, Brno, p 97
152. Kowalski E, Ludwig H, Tauscher B (1992) Deutsche Lebensmittel-Rundschau 88:74
153. Kowalski E, Ludwig H, Tauscher B (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 341–343
154. Butz P, Heinisch O, Tauscher B (1993) Eur High Pressure Research Grp, 31th Annual Meeting, Belfast 30.8.–3.9.
155. Clever V, Tauscher B (1994) Eur High Pressure Research Grp, 32th Annual Meeting, Brno, ISBN 80–85867–25–7, p 99
156. Horie Y, Kimura K, Ida M, Yoshida Y, Ohki K (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 236–242
157. Watanabe M, Aral E, Kumeno K, Honma K (1991) Agric Biol Chem 55:2175
158. Ito S (1992) Shokuhin Kogyo (Food Industry) 37:38
159. Sumitani H, Suekane S, Nakatani A, Tatsuka K (1994) J Agric Food Chem 42:785
160. Butz P, Koller WD, Braun M, Tauscher B (1993) In: Gesellschaft Deutscher Chemiker, 24. Hauptversammlung, Kurzreferate, ISBN 3–527–29083–4, p 321
161. Kinugasa H, Takeo T, Fukumoto K, Shinno T, Ishihara M (1992) In: Hayashi R (ed) High pressure bioscience and food science. San-Ei Shuppan, Kyoto, pp 243–248
162. Ifuku Y, Takahashi Y (1990) In: Hayashi R (ed) Pressure-processed food, research and development. San-Ei Publ Co, Kyoto, pp 165–177
163. Takahashi Y, Ohta H, Yonei H, Ifuku Y (1993) Int J Food Sci Technol 28:95
164. Tamaki Y (1992) In: Hayashi R (ed) High Pressure Bioscience and Food Science. San-Ei Shuppan, Kyoto, pp 403–407
165. Parish ME, Floridan U (1994) Institute of Food Technologists Annual Meeting, Atlanta June 25–29, Session 42–1
166. Deuchi T, Hayashi R (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 353–355
167. Kanda Y, Aoki M, Kosugi T (1992) Nippon Shokuhin Kogyo Gakkaishi 39:608
168. Studer D, Hennecke H, Müller M (1992) Planta 188:155
169. Murakami T, Kimura I, Yamagishi T, Yamashita M, Sugimoto M, Satake M (1992) In: Balny C, Hayashi R, Heremans K, Masson P (eds) High pressure and biotechnology. Colloques INSERM 224, J Libbey, London, pp 329–331
170. Honma K, Makino T, Kumeno K, Watanabe M (1993) Biosci Biotech Biochem 57:1091