ELSEVIER

Trends in Food Science & Technology 9 (1998) 197–203

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Effects of high pressure on enzymes related to food quality

M. Hendrickx, L. Ludikhuyze, I. Van den Broeck and C. Weemaes

Department of Food and Microbial Technology, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, K. Mercierlaan 92, B-3001 Heverlee, Belgium (Tel: 32-16-32.15.85; Fax: 32-16-32.19.60; e-mail: marc.hendrickx@agr.kuleuven.ac.be; linda.ludikhuyze@agr.kuleuven.ac.be; ilse.den@agr.kuleuven.ac.be; carla.weemaes@agr.kuleuven.ac.be)

High-pressure processing has potential for food preservation purposes because it can inactivate microorganisms and enzymes. In order to implement this new technology in the food industry, we need to understand the mechanism and kinetics of pressure-induced degradation/denaturation/(in)activation of several food compounds (e.g. microorganisms, enzymes, nutrients) and the way in which the degradation/denaturation/(in)activation is influenced by other parameters (e.g. temperature, pH). This review is concerned with the factors influencing the effect of high pressure on enzymes related to food quality.

Introduction

Consumer demand for minimally processed, microbiologically safe, stable food products that are additive free, has stimulated the interest of food companies in high-pressure processing [1, 2]. Academic research laboratories and food companies have initiated research on the effects of high pressure on several food constituents in order to evaluate the effect of this new technology on food safety and quality. Whereas high-pressure research initially was mainly a qualitative trial and error process, it is today characterized by a more systematic fundamental approach in which attention is given to kinetic aspects and to specific products.

Vegetative cells, including yeasts and moulds, are rather pressure sensitive; i.e. they can be inactivated by pressures of ~300–600 MPa [3, 4]. Bacterial spores, on the other hand, are highly pressure resistant, since pressures exceeding 1200 MPa may be needed for their inactivation [3, 5]. Preservation of acid foods ($pH \leq 4.6$) is, therefore, the most likely application of high-pressure processing *per se* [6]. Sterilization of low-acid foods ($pH > 4.6$), on the other hand, will most probably rely on a combination of high-pressure processing and other (mild) treatments. For both pasteurization and sterilization processes, combined pressure–temperature treatments are frequently regarded as most appropriate [1, 4].

An advantage of the new technology is that food quality characteristics such as flavour and vitamins are unaffected or only minimally altered by high-pressure processing at room temperature [7–9]. On the other hand, enzymes related to food quality can be deactivated by pressure [10]. The pressure needed strongly depends on the enzyme: some enzymes can be deactivated at room temperature by a few hundred MPa, while others can withstand 1000 MPa [11–13]. Because of the extreme pressure stability of some food quality enzymes, combined processes (e.g. pressure and temperature) might be necessary for enzyme inactivation at industrially relevant pressures [1, 7]. However, in a few cases, enzyme activation due to pressure treatment alone has been observed [13, 14].

The ability of high pressure to inactivate microorganisms and food quality enzymes and to leave other quality attributes intact has encouraged Japanese food industries and recently also an American food company to introduce high-pressure preserved foods on the market [15, 16]. Most such products are acid foods, such as fruit juices and jams. In addition to food preservation, high-pressure processing opens up the possibility of producing foods with novel texture (e.g. meat, fish, dairy products) [17]. The modification of food protein functionality by high pressure has recently been reviewed by Messens *et al.* [18].

0924-2244/98/$—see copyright statement.
PII: S0924-2244(98)00039-9

The effect of high pressure on proteins: fundamental aspects

General aspects of protein structure

In proteins, four levels of structure can be distinguished. The primary structure, which is defined as the amino acid sequence and the location of disulphide bonds (if any), gives a complete description of the covalent bonds of a protein. The secondary structure refers to the way in which the polypeptide chain forms α-helices or β-sheets by intra- or intermolecular hydrogen bonds. The tertiary structure describes how the secondary structure domains fold into a three-dimensional configuration as a consequence of noncovalent interactions between amino acid side chains. The quaternary structure refers to the spatial arrangement of subunits, held together by noncovalent bonds between the polypeptide subunits (multimeric proteins).

Proteins are delicate structures, maintained by interactions within the protein chain (determined by the amino acid sequence) and by interactions with the surrounding solvent [19]. Changes in external factors, such as pressure and temperature, can perturb the subtle balance of intramolecular and solvent–protein interactions and can, therefore, lead to (complete) unfolding/denaturation of the polypeptide chain.

Pressure-induced changes in protein structure

Structural rearrangements taking place in the protein upon pressurization are governed by the principle of Le Chatelier, which states that processes associated with a volume decrease are encouraged by pressure increases, whereas processes involving a volume increase are inhibited by pressure increases. The volume decrease accompanying denaturation ($\sim$ 30–80 ml/mol [20]) arises from the formation or rupture of noncovalent bonds (i.e. changes in conformational volume) and from rearrangements of solvent molecules (i.e. changes in solvation volume).

Regarding changes in conformational volume, at least at relatively low temperature, covalent bonds are almost unaffected by high pressure, and hence the primary structure will remain intact during pressure treatment [7, 17]. Secondary structure changes, on the other hand, occur at very high pressure and these lead to irreversible denaturation [21, 22]. This might be explained by the fact that hydrogen bonds, which are responsible for maintaining the helical structure of peptides, are enhanced at low pressures and are only ruptured at very high pressure [23]. The rupture of ionic bonds is strongly affected by pressure increases [24, 25]. The effect of pressure on hydrophobic interactions is rather complex. Heremans [24] stated that opinions on the effect of pressure on hydrophobic interactions are as divergent as the opinions on the nature of hydrophobic interactions themselves. Significant tertiary structure changes (maintained chiefly by hydrophobic and ionic interactions) are mostly observed in excess of 200 MPa [22]. Multimeric proteins (quaternary structure), held together by noncovalent bonds, are dissociated by comparatively low pressures (< 150 MPa). The exposure to solvent of protein surfaces, which formerly interacted with each other, results in the binding of water molecules, causing a volume decrease in the system; i.e. a pressure increase shifts the equilibrium towards monomerization [26]. In some cases, pressures exceeding 150 MPa induce reassociation of the dissociated subunits [27]. From the above, it is clear that, contrary to thermal treatments, where covalent as well as noncovalent bonds are affected, high-pressure processing at room temperature only disrupts relatively weak chemical bonds (hydrogen bonds, hydrophobic bonds, ionic bonds). As to the effect of high pressure on chemical bonds at high temperature, very little information is available in the literature. Alterations in protein conformation can bring about changes in the functional properties of food proteins and hence high-pressure treatment of foods can be used to create new products with a unique texture or taste [18].

Changes in solvation volume are mainly caused by pressure-induced ionization, changes in solvent exposure of amino acid side chains and peptide bonds, and diffusion of water into cavities located in the hydrophobic core of the protein [28]. Thus, the volume decrease results from water binding around charged groups, water structuring around newly solvent-exposed apolar groups (hydrophobic hydration), and solvation of polar groups through hydrogen bonding [29]. Low and Somero [30] stated, however, that the exposure of hydrophobic groups to water can lead to volume increases or decreases, depending on the types and concentrations of adjacent hydrophobic groups. Localized unfolding and subunit dissociation can result in the unmasking of buried groups that are able to pair with other newly exposed groups. This process can lead to aggregation [21].

In general, pressures above 300 MPa cause irreversible protein denaturation at room temperature, while lower pressures result in reversible changes in protein structure [7, 24]. A lowering of the denaturation pressure with both decreases (cold denaturation) and increases in temperature has been observed [12, 31, 32].

Effect of pressure on enzymes

Enzymes are a special class of proteins in which biological activity arises from an active site, brought together by the three-dimensional configuration of the molecule. Even small changes in the active site can lead to a loss of enzyme activity [33]. Since protein denaturation is associated with conformational changes, it can change the functionality of the enzyme (e.g. increase or loss of biological activity, change in substrates pecificity).

Effects of high pressure on enzymes may be divided in two classes. In the first, comparatively low pressures (~ 100 MPa) have been shown to activate some enzymes [14, 34, 35]. This stimulation effect is, however, only observed for monomeric enzymes. Much higher pressures, on the other hand, generally induce enzyme inactivation [34]. With regard to pressure inactivation, Miyagawa *et al.* [36] distinguished four groups of enzyme, based on loss and recovery of activity: (i) completely and irreversibly inactivated, (ii) completely and reversibly inactivated, (iii) incompletely and irreversibly inactivated, and (iv) incompletely and reversibly inactivated. Both high-pressure induced activation and inactivation are relevant to food quality. In addition to conformational changes [14], enzyme activation can arise from pressure-induced decompartmentalization [37, 38]. In intact tissues, enzymes and substrate are often separated by compartmentalization, which can be destroyed upon application of low pressure [35, 37]. Pressure-induced membrane damage and the resulting leakage of enzyme and substrate result in enzyme-substrate contact. The enzymatic reaction resulting from this contact can, in turn, be accelerated or decelerated by pressure, depending on the reaction volume of the enzyme catalysed reaction [39]. Concerning pressure inactivation, there seems to be a minimum pressure below which no or little enzyme inactivation occurs. When pressure exceeds this value, enzyme inactivation (within a specified time interval) increases until completed at a certain pressure. This pressure inactivation range is strongly dependent on the type of enzyme, pH, medium composition, temperature etc. [7, 22]. For some enzymes (e.g. trypsin, EC 3.4.4.4; chymotrypsin, EC 3.4.4.5; chymotrypsinogen), there seems to be a maximum pressure above which further pressure increase does not result in additional inactivation [34, 40]. This has been attributed to an enzyme portion that is irreversibly converted to the inactive form, while a fraction is converted to a very pressure-resistant form. Upon pressure release, the pressure-resistant fraction reverts to the equilibrium state, while the irreversibly inactivated enzyme remains unchanged [34].

It has been suggested that the efficiency of high-pressure enzyme inactivation is improved by applying pressure cycles. Successive applications of high pressure resulted in higher inactivation of many enzymes (trypsin; chymotrypsin; pepsin, EC 3.4.23.1; *Bacillus subtilis* α-amylase, EC 3.2.1.1); i.e. the activity retention after a multi-cycle process was lower than that of a single-cycle process with the same total duration [34, 40, 41]. However, repeated steps of pressure build-up and release appeared to have no effect on pectin methyl esterase [42] (EC 3.1.1.11) [Crelier *et al.*, (1995) *High pressure for the inactivation of enzymes in food products. Thermal and HHP treatment of tomato pectin methyl esterase* (poster presentation at 'International symposium on high pressure effects on foods', Budapest, Hungary)] and chymotrypsin [34]. For trypsin and chymotrypsin, it has been reported that successive pressure treatments result in a higher degree of inactivation only when pressures above the minimum pressure are applied [34].

The effect of high pressure on food quality enzymes

Kinetics of pressure inactivation

Up to now it has been believed that only a combination of high pressure and moderate temperature elevation will lead to microbiologically safe products [43] and economically feasible processes [2]. The time course of isobaric and/or isothermal enzyme inactivation due to pressure and/or temperature can often be described by nth-order, first order or fractional conversion models [see Box 1]. The latter model, which is a special case of a first order model, is used when a resistant fraction persists after the inactivation process. Kinetic parameters describing the course of inactivation, and pressure and temperature dependence of the inactivation rate constant [see Box 1] are of key importance for design and optimization of combined high pressure/temperature processing for the preservation of food products.

A complete kinetic characterization of pressure- and/or temperature-induced enzyme inactivation has recently been published by Ludikhuyze *et al.* [41, 44] for the model enzyme system *Bacillus subtilis* α-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; 25–82°C) and a pressure–temperature kinetic diagram was constructed. This is a two-dimensional diagram indicating possible synergistic and antagonistic effects of pressure and temperature. An example of such a pressure–temperature kinetic diagram is shown in Fig. 1. Based on the inactivation rate constants, activation volumes and activation energies were calculated, and a kinetic model was proposed. This model was validated under dynamic pressure–temperature conditions. Analogous detailed, quantitative, kinetic studies of enzyme (in)activation need to be carried out for food quality enzymes in order to implement combined pressure–temperature technology in the food industry. In the following paragraphs, available (kinetic) data for enzymes affecting texture, flavour or colour of foods are summarized.

Pectin methyl esterase

Pectin methyl esterase (PME; EC 3.1.1.11) is responsible for cloud destabilization of (orange) juices, gelation of concentrates and consistency loss of (tomato) products. PME is inactivated by heat in conventional preservation processes, which leads to detrimental effects on flavour, colour and nutritional quality [42]. High-pressure processing of orange juice can result in a

Box 1. Kinetic models to describe the pressure–temperature inactivation of enzymes

Course of inactiviation:

n-th order inactivation model	$A = (A_0 + (n-1)kt)^{\frac{1}{1-n}}$	1
first-order inactivation model	$A = A_0 \exp(-kt)$	2
fractional conversion model	$A = A_\infty + (A_0 - A_\infty)\exp(-kt)$	3

P-dependence of k:

Theory of Eyring	$k = k_{\text{atm}} \exp\left(\frac{-V_a}{RT}(p - P_{\text{ref}})\right)$	4

T-dependence of k:

Arrhenius equation	$k = k_{\text{ref}} \exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right)$	5

A = enzyme activity at time t
A_0 = enzyme activity at time zero
A_∞ = enzyme concentration at equilibrium
E_a = activation energy (kJ/mol)
k = inactivation rate constant at temperature T and pressure P (min^{-1})(assumes $n = 1$)
k_{atm} = inactivation rate constant at temperature T and atmospheric pressure (min^{-1})(assumes $n = 1$)
k_{ref} = inactivation rate constant at pressure P and reference temperature (min^{-1})(assumes $n = 1$)
n = reaction order
P = pressure (MPa)
P_{ref} = reference pressure (0.1 MPa)
R = gas constant (8.314 J/mol K)
t = time (min)
T = absolute temperature (K)
T_{ref} = absolute reference temperature (K)
V_a = activation volume (cm^3/mol)

commercial stable product with higher quality [45]. It has been reported that high-pressure treatments of ~ 600 MPa can partially (up to 90%) and irreversibly inactivate orange PME [42, 45], which does not reactivate during storage and transportation [45]. A kinetic model of the pressure inactivation of orange PME has not yet been proposed. Tomato PME seems to be more pressure resistant and its inactivation seems to follow first-order kinetics [10] [Crelier *et al.*, (1995) *High pressure for the inactivation of enzymes in food products. Thermal and HHP treatment of tomato pectin methyl esterase* (poster presentation at 'International symposium on high pressure effects on foods', Budapest, Hungary)]. Tomato PME is less pressure stable in the presence of Ca ions or in citric acid buffer (pH 3.5–4.5) than in water, and, its pressure stability decreases with decreasing pH. Moreover, the absolute value of the activation volume decreases with increasing pH (about −28 to −40 cm^3/mol) and upon addition of Ca ions. At temperatures where the enzyme inactivates at atmospheric pressure, an antagonistic effect of pressure and temperature was observed, i.e. inactivation was slower at elevated pressure than at atmospheric pressure. The antagonistic effect was less pronounced in citric acid buffer (pH 3.8–4.5) or in the presence of $CaCl_2$ than in water.

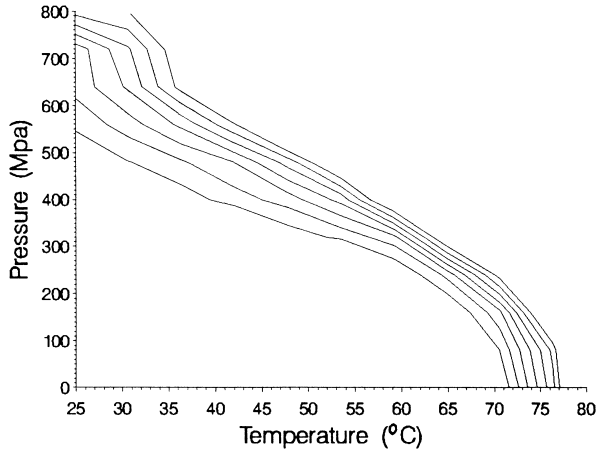

Fig. 1. Pressure–temperature kinetic diagram of *Bacillus subtilis* α-amylase (15 mg/ml in 0.01 M Tris HCl at pH 8.6). Range: k = 0.01 min^{-1} (lower line)— k = 0.07 min^{-1} (upper line). Reproduced with permission from [44].

At low pressures and elevated temperatures (59–60°C), activation of tomato PME was observed. In the absence or presence of Ca ions, the optimal pressures for enzyme activation were 100 and 400 MPa, respectively. Pressure-induced activation was more pronounced in the presence of Ca ions (Van den Broeck *et al.*, unpublished). Pressure-induced activation of PME in freshly squeezed orange juice was also noted by Cano *et al.* [13] in the case of treatments at room temperature and 200–400 MPa.

Peroxidase

In vegetables, peroxidase (POD; EC 1.11.1.7) induces negative flavour changes during storage. The enzyme, which is the most heat-stable vegetable enzyme, is at least in some cases extremely pressure resistant. For green beans, a treatment of 900 MPa for 10 min at room temperature was needed to cause an 88% reduction of POD activity. A combination with temperature treatments enhanced the inactivating effect at 600 MPa, but no significant differences were detected at 700 MPa.

Increasing the pressurization time seemed to have no significant effect [46]. In strawberry puree, POD was increasingly inactivated up to 300 MPa for treatments at 20°C for 15 min. Above 300 MPa, POD activity was slightly increased. Above 45°C, a decrease in activity was found for all pressures (50–400 MPa). At room temperature, the activity of orange POD decreased continuously up to 400 MPa (processing time 15 min). The highest inactivation rate (50%) was found at 32°C. High-pressure treatments at 32–60°C adversely increased POD activity in orange juice [13].

In the case of lactoperoxidase, a verdoperoxidase, the barotolerance in the temperature range 10–30°C was strongly dependent on the medium. Pressure inactivation was much more pronounced in Tris buffer (pH 7) than in milk. In Tris buffer, the initial activity was reduced by 70% upon treatment at 600 MPa, for 2 min at 25°C [10].

Lipase

Lipase (EC 3.1.1.3) is responsible for hydrolysis of animal and vegetable fats and oils. Large differences in the pressure stability of lipase have been reported in literature. According to Macheboeuf and Basset [11] lipase is stable for up to 1100 MPa, whereas, according to Seyderhelm *et al.* [10], inactivation is noticeable at about 600 MPa.

Lipoxygenase

Lipoxygenase (LOX; EC 1.13.1.13) catalyses the oxygenation of fatty acids containing a cis,cis,1,4-pentadiene system into the corresponding hydroperoxides. In a secondary reaction, strongly odorous scission products are formed. Several combinations of high pressure (up to 750 MPa) and temperature (0–75°C) for a period of 5 min have been used for the inactivation of soybean LOX in Tris buffer, pH 8.3. A pressure–temperature phase diagram was constructed based on activity loss [12].

Pressure inactivation of soybean LOX could be accurately described by a first-order kinetic model and the kinetic parameters seemed to be strongly dependent on environmental conditions. Pressure resistance of the enzyme increased with increasing enzyme concentration and decreased with decreasing pH in the range 9–5.4. Furthermore, the pressure stability was much higher in Tris buffer than in McIllvaine buffer. Flushing with CO_2 strongly reduced pressure stability of soybean LOX (Ludikhuyze *et al.*, unpublished). A complete kinetic study has been performed for soybean LOX (0.4 mg/ml, 0.01 M Tris HCl buffer, pH 9). Inactivation rate constants for 60 combinations of constant pressure (0–650 MPa) and temperature (10–65°C) were used to construct a pressure–temperature kinetic diagram. Based on the diagram developed, it was concluded that soybean LOX is most pressure stable around room temperature. Both temperature increases and decreases enhanced the inactivation effect. The pressure dependence of the inactivation rate constant was rather high (around $-70\ cm^3/mol$) and about constant in the temperature range 10–40°C, whereas in the temperature range 40–65°C, the pressure sensitivity was strongly reduced (Ludikhuyze *et al.*, unpublished).

Polyphenoloxidase

Polyphenoloxidase activity (PPO; EC 1.14.18.1) results in enzymatic browning of damaged fruits and vegetables. Because of the brown coloration and the concomitant changes in appearance and organoleptic properties, inactivation of PPO is highly desirable. High pressure can be used as an alternative to high temperature for the irreversible inactivation of PPO. Mushroom and potato PPO are very pressure stable, since treatments at $\sim$ 800–900 MPa are required for activity reduction [38, 47, 48]. Grape, strawberry, apricot and apple PPO seem to be more pressure sensitive. Apricot, strawberry and grape PPO could be inactivated by pressures exceeding $\sim$ 100, 400 and 600 MPa, respectively [35, 49]; depending on pH, pressures of 100–700 MPa were needed for the inactivation of apple PPO [50]. For several PPO enzymes, it has been reported that pressure-induced inactivation proceeds faster at lower pH [35, 48]. In addition to pH, pressure inactivation is influenced by the addition of salts, sugars or other compounds. The pressure inactivation of apple PPO is enhanced by the addition of $CaCl_2$ [35] and that of mushroom PPO is enhanced in the presence of 50 mM benzoic acid or 5 mM glutathione [48]. The sensitising effect of glutathione was suggested to be due to an interaction with a disulphide bond of the enzyme.

For avocado PPO, a detailed study of the combined effect of pressure (0.1–900 MPa) and temperature (25–77.5°C) was made. The inactivation of the enzyme at room temperature was observed at $\sim$800–900 MPa and there was an antagonistic effect of pressure and temperature at pressures below 250 MPa and temperatures exceeding 62.5°C. Activation energies seemed to increase with increasing pressure, as was observed for mushroom PPO [48]. Activation volumes were only constant at pressures exceeding 300 MPa. Based on the results obtained, a mathematical model predicting the combined effect of pressure and temperature on avocado PPO has been formulated (Weemaes *et al.*, unpublished).

In addition to inactivation of PPO at high pressure, pressure-induced activation at low pressure has been reported for apple [35, 50], onion [37], pear [14] and strawberry PPO [13].

Conclusion

From the examples above, the role of pressure, temperature and medium on enzyme (in)activation is clear.

Since pressure resistance of enzymes is not related to thermal resistance, knowledge previously amassed in the thermal area cannot be transposed to the pressure (–temperature) area. Moreover, enzyme activation at low pressure can lead to food quality problems (e.g. colour). Enzyme activation can, on the other hand, be used to create new functionality.

From an engineering point of view, kinetic data of food quality enzymes are indispensable for design, evaluation and optimization of processes based on the effect of pressure (and temperature). Early attempts at such a quantitative approach have been made, but more extensive kinetic data on the pressure (–temperature) (in)activation of food quality enzymes are needed. There remains a need for the modelling of combined pressure-temperature (in)activation processes.

Acknowledgements

The authors would like to thank NFSR (National Fund for Scientific Research), IWT (Vlaams Instituut voor de bevordering van het Wetenschappelijk-Technologisch Onderzoek in de Industrie), K.U.Leuven Research Council and the European Commission for financial support.

References

1 Farr, D. (1990) 'High Pressure Technology in the Food Industry' in *Trends Food Sci. Technol.* 1, 14–16

2 Mertens, B. (1992) 'Under Pressure' in *Food Manufacture* 67, 23–24

3 Knorr, D. (1995) 'Hydrostatic Pressure Treatment of Food : Microbiology' in *New Methods of Food Preservation* (Gould, G.W., ed.), pp. 159–175, Blackie Academic and Professional

4 Patterson, M.F., Quinn, M., Simpson, R. and Gilmour, A. (1995) 'Effects of High Pressure on Vegetative Pathogens' in *High Pressure Processing of Foods* (Ledward, D.A., Johnston, D.E., Earnshaw, R.G. and Hasting, A.P.M., eds), pp. 47–63, Nottingham, University Press

5 Larson, W.P., Hartzell, T.B. and Diehl, H.S. (1918) 'The Effect of High Pressures on Bacteria' in *J. Infect. Dis.* 22, 271–279

6 Lechowich, R.V. (1993) 'Food Safety Implications of High Hydrostatic Pressure as a Food Processing Method' in *Food Technol.* 47, 170–172

7 Cheftel, J.-C. (1991) 'Applications des Hautes Pressions en Technologie Alimentaire' in *Ind. Aliment. Agric.* 108, 141–153

8 Kimura, K., Ida, M., Yoshida, Y., Ohki, K., Fukumoto, T. and Sakui, N. (1994) 'Comparison of Keeping Quality Between Pressure Processed Jam: Changes in Flavor Components, Hue and Nutrients During Storage' in *Biosci. Biotech. Biochem.* 58, 1386–1391

9 Yen, G.C. and Lin, H.T. (1996) 'Comparison of High Pressure Treatment and Thermal Pasteurisation Effects on the Quality and Shelf-life of Guava Puree' in *Int. J. Food Sci. Technol.* 31, 205–213

10 Seyderhelm, I., Boguslawski, S., Michaelis, G. and Knorr, D. (1996) 'Pressure Induced Inactivation of Selected Food Enzymes' in *J. Food Sci.* 61, 308–310

11 Macheboeuf, M.A. and Basset, J. (1934) 'Die Wirkung sehr hoher Drucke auf enzyme' in *Ergebnisse der Enzymforschung* 3, 303–308

12 Heinisch, O., Kowalski, E., Goossens, K., Frank, J., Heremans, K., Ludwig, H. and Tauscher, B. (1995) 'Pressure Effects on the Stability of Lipoxygenase : Fourier Transform-Infrared Spectroscopy (FT-IR) and Enzyme Activity Studies' in *Z. Lebensm Unters Forsch.* 201, 562–565

13 Cano, M.P., Hernandez, A. and De Ancos, B. (1997) 'High Pressure and Temperature Effects on Enzyme Inactivation in Strawberry and Orange Products' in *J. Food Sci.* 62, 85–88

14 Asaka, M., Aoyama, Y., Ritsuko, N. and Hayashi, R. (1994) 'Purification of a Latent Form of Polyphenoloxidase from La France Pear Fruit and its Pressure-activation' in *Biosci. Biotech. Biochem.* 58, 1486–1489

15 Cheftel, J.-C. (1995) 'Hautes Pressions, Inactivation Microbienne et Conservation des Aliments' in *C.R. Agric. Fr.* 81, 13–38

16 Mermelstein, N.H. (1997) 'High-pressure Processing Reaches the U.S. Market' in *Food Technol.* 51, 95–96

17 Hayashi, R. (1989) 'Application of High Pressure to Food Processing and Preservation: Philosophy and Development' in *Engineering and Food* (Spiess, W.E.L. and Schubert, eds), pp. 815–826, Applied Sci., England

18 Messens, W., Van Camp, J. and Huyghebaert, A. (1997) 'The Use of High Pressure to Modify the Functionality of Food Proteins' in *Trends Food Sci. Technol.* 8, 107–112

19 Jaenicke, R. (1991) 'Protein Stability and Molecular Adaptation to Extreme Conditions' in *Eur. J. Biochem.* 202, 715–728

20 Silva, J.L. and Weber, G. (1993) 'Pressure Stability of Proteins' in *Ann. Rev. Phys. Chem.* 44, 89–113

21 Masson (1992) 'Pressure Denaturation of Proteins' in *High Pressure and Biotechnology* (Balny, C., Hayashi, R., Heremans, K. and Masson, P., eds), pp. 89–99, Colloque INSERM vol 224

22 Balny, C. and Masson, P. (1993) 'Effects of High Pressure on Proteins' in *Food Rev. Int.* 9, 611–628

23 Suzuki, K. and Taniguchi, Y. (1972) 'Effect of Pressure on Biopolymers and Model Systems' in *Symposia of the Society for Experimental Biology; the Effects of Pressure on Organisms*, pp. 103–121, Cambridge University Press

24 Heremans, K. (1993) 'The Behaviour of Proteins Under Pressure' in *High Pressure Chemistry, Biochemistry and Material Science* (Winter, R. and Jonas, J, eds), pp. 443–469, Kluwer Academic Publishers

25 Gross, M. and Jaenicke, R. (1994) 'Proteins Under Pressure : the Influence of High Hydrostatic Pressure on Structure, Function and Assembly of Proteins and Protein Complexes' in *Eur. J. Biochem.* 221, 617–630

26 Penniston, J.T. (1971) 'High Hydrostatic Pressure and Enzymic Activity : Inhibition of Multimeric Enzymes by Dissociation' in *Arch. Biochem. Biophys.* 142, 322–332

27 Ohmiya, K., Kajino, T., Shimizu, S. and Gekko, K. (1989) 'Effect of Pressure on the Association State of Enzyme-Treated Caseins' *Agric. Biol. Chem.* 53, 1–7

28 Carter, J.V., Knox, D.G. and Rosenberg, A. (1978) 'Pressure Effects on Folded Proteins in Solution : Hydrogen Exchange at Elevated Pressures' in *J. Biol. Chem.* 253, 1947–1953

29 Heremans, L. and Heremans, K. (1989) 'Raman Spectroscopic Study of the Changes in Secondary Structure of Chymotrypsin : Effect of pH and Pressure on the Salt Bridge' in *Biochim. Biophys. Acta.* 999, 192–197

30 Low, P.S. and Somero, G.N. (1975) 'Pressure Effects on Enzyme Structure and Function in vitro and Under Simulated in vivo Conditions' in *Comp. Biochem. Physiol.* 52B, 67–74

31 Suzuki, K. (1958) 'Studies on the Denaturation of Egg Albumin Under High Pressure' in *Rev. Phys. Chem. Jpn.* 28, 24–30

32 Suzuki, K. (1960) 'Studies on the Kinetics of Protein Denaturation Under High Pressure' in *Rev. Phys. Chem. Jpn.* 29, 49–55

33 Tsou, C.-L. (1986) 'Location of the Active Sites of Some Enzymes in Limited and Flexible Molecular Regions' in *Trends Biochem. Sci.* 11, 427–429

34 Curl, L. and Jansen, E.F. (1950) Effect of High Pressures on Trypsin and Chymotrypsin' in *J. Biol. Chem.* 184, 45–54
35 Jolibert, F., Tonello, C., Sagegh, P. and Raymond, J. (1994) 'Les Effets des Hautes Pressions sur la Polyphénol Oxydase des Fruits' in *Bios Boissons* 251, 27–35
36 Miyagawa, K., Sannoe, K. and Suzuki, K. (1964) 'Studies on Taka-Amylase A Under High Pressure Treatment ; Part II : Recovery of Enzymic Activity of Pressure Inactivated Taka-Amylase A and its Enhancement by Retreatment at Moderate Pressure' in *Arch. Biochem. Biophys.* 106, 467–474
37 Butz, P., Koller, W.D., Tauscher, B. and Wolf, S. (1994) 'Ultra-high Pressure Processing of Onions: Chemical and Sensory Changes' in *Lebensm. -Wiss u. Technol.* 27, 463–467
38 Gomes, M.R.A. and Ledward, D.A. (1996) 'Effect of High Pressure Treatments on the Activity of Some Polyphenoloxidases' in *Food Chem.* 56, 1–5
39 Morild, E. (1981) 'The Theory of Pressure Effects on Enzymes' in *Adv. Protein Chem.* 34, 93–166
40 Curl, L. and Jansen, E.F. (1950) 'The Effect of High Pressures on Pepsin and Chymotrypsinogen' in *J. Biol. Chem.* 185, 716–723
41 Ludikhuyze, L.R., Van den Broeck, I., Weemaes, C.A. and Hendrickx, M.E. (1997) 'Kinetic Parameters for Pressure–Temperature Inactivation of *Bacillus subtilis* α-amylase Under Dynamic Conditions' in *Biotechol. Progr.* 13, 617–623
42 Irwe, S. and Olsson, I. (1994) 'Reduction of Pectinesterase Activity in Orange Juice by High Pressure Treatment' in *Minimal Processing of Foods and Process Optimization : an Interface* (Singh, R.P. and Oliveira, F., eds), pp. 35–42, CRC Press
43 Gould, G.W. (1973) 'Inactivation of Spores in Food by Combined Heat and Hydrostatic Pressure' in *Acta Alimentaria* 2, 377–383
44 Ludikhuyze, L.R., Van den Broeck, I., Weemaes, C.A., Herremans, C.H., Van Impe, J.F., Hendrickx, M.E. and Tobback, P.P (1997) 'Kinetics for Isobaric–Isothermal Inactivation of *Bacillus subtilis* α-amylase' in *Biotechnol. Progr.* 13, 532–538
45 Ogawa, H., Fukuhisa, K., Kubo, Y. and Fukumoto, H. (1990) 'Pressure Inactivation of Yeasts, Molds and Pectinesterase in Satsuma Mandarin Juice : Effects of Juice Concentration, pH and Organic Acids, and Comparison with Heat Sanitation' in *Agric. Biol. Chem.* 54, 1219–1225
46 Quaglia, G.B., Gravina, R., Paperi, R. and Paoletti F. (1996) 'Effect of High Pressure Treatments on Peroxidase Activity, Ascorbic Acid Content and Texture in Green Peas' in *Lebensm. -Wiss. u. -Technol.* 29, 552–555
47 Eshtiaghi, M.N., Stute, R. and Knorr, D. (1994) 'High Pressure and Freezing Pretreatment Effects on Drying, Rehydration, Texture and Color of Green Beans, Carrots and Potatoes' in *J. Food Sci.* 59, 1168–1169
48 Weemaes, C.A., De Cordt, S.V., Ludikhuyze, L.R., Van den Broeck, I., Hendrickx, M.E. and Tobback, P.P. (1997) 'Influence of pH, Benzoic Acid, EDTA, and Glutathione on the Pressure and/or Temperature Inactivation Kinetics of Mushroom Polyphenoloxidase' *Biotechnol. Progr.* 13, 25–32
49 Amati, A., Castellari, M., Matricardi, L., Arfelli, G. and Carpi, G. (1996) 'Modificazione Indotte in Mosti d'uva da Trattamenti Con Alte Pressioni Idrostatische (Effects of High Pressure on Grape Musts Composition' *Industrie delle Bevande* 25, 324–328
50 Anese, M., Nicoli, M.C., Dall'aglio, G. and Lerici, C. (1995) 'Effect of High Pressure Treatments on Peroxidase and Polyphenoloxidase Activities' in *J. Food Biochem.* 18, 285–293