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**LES HAUTES PRESSIONS HYDROSTATIQUES ET LEURS
APPLICATIONS EN SCIENCE DES ALIMENTS,
Une étude : des phénomènes thermiques liés à la compression, des
cinétiques d'inactivation microbienne, des traitements en pression
pulsée et en pression de dioxyde de carbone.**

**HIGH HYDROSTATIC PRESSURE (HHP) APPLICATIONS
IN FOOD SCIENCE :**

**a study on compression heating, Microbial inactivation kinetics,
pulsed pressure and high pressure carbon dioxide treatments.**

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RESUME EN FRANÇAIS

Les consommateurs recherchent de plus en plus des aliments de haute qualité en termes d'arômes, de goût, de sécurité microbienne et sans additifs chimiques.

L'application de technologies émergentes comme les Hautes Pressions Hydrostatiques (HPH), les champs électriques pulsés... peut être utilisée pour remplacer, modifier ou améliorer les techniques conventionnelles de traitement alimentaire.

Les hautes pressions hydrostatiques, en tant que traitement non-thermique, présentent un certain nombre d'avantages. L'un d'entre eux résulte de deux facteurs : la non-utilisation de la chaleur et la faible énergie développée par la pression. En conséquence les caractéristiques sensorielles et nutritionnelles (résultant principalement de molécules caractérisées par des liaisons fortes) demeurent pratiquement non affectées. Ceci conduit ainsi à des produits de plus grande qualité que ceux issus des procédés conventionnels.

Quelques publications ont aussi mis en évidence des nouvelles possibilités d'application des traitements hautes pressions : telles que l'élimination du caractère allergène des protéines des aliments (Nogami et al. 2006) ou la meilleure digestibilité de l'amidon traité sous haute pression en présence de glucoamylases (Takahashi et al. 1994).

Durant ces deux dernières décades, les connaissances impliquant l'application des hautes pressions hydrostatiques aux procédés de l'agro-alimentaire ont suscité un grand intérêt du fait de caractéristiques spécifiques :

- (i) l'accroissement de la qualité des produits,
- (ii) la modification des propriétés physiques et fonctionnelles des systèmes d'aliments,
- (iii) l'utilisation des transitions de phases anormales de l'eau sous haute pression : ie. l'abaissement du point de solidification lors de l'accroissement de pression (ce

phénomène étant lié à la faible compacité de la glace I – variété structurale à pression normale – par rapport à l'eau liquide).

La technologie des Hautes Pressions Hydrostatiques dans les procédés de traitements des aliments peut être résumée au travers de cinq principales caractéristiques :

- 1- le développement des procédés hautes pressions à température ambiante ou à température négatives,
- 2- la transmission instantanée de la pression dans tout le système quelles que soient la taille et la géométrie de l'échantillon,
- 3- l'inactivation microbienne par une méthode non-thermique et l'utilisation possible d'additifs afin d'accroître la qualité globale des aliments,
- 4- la réduction, – dans certains cas – du caractère allergénique de certains aliments ou de l'accroissement de leur digestibilité, pouvant conduire à des applications nutritionnelles spécifiques,
- 5- la création de nouveaux aliments avec des propriétés organoleptiques et fonctionnelles particulières.

L'objectif de cette thèse étant d'apporter une contribution scientifique à l'application des Hautes Pressions Hydrostatiques aux procédés de traitement des aliments, le manuscrit a été présenté sous la forme de cinq Chapitres.

Le **Chapitre I** relate un bref historique du développement des hautes pressions dans le domaine des Biosciences.

Le **Chapitre II** résume les principaux effets des Hautes Pressions Hydrostatiques (HPH) sur les constituants des aliments (y compris les micro-organismes contaminants).

Le **Chapitre III** décrit les Matériels utilisés et les Méthodes mises en œuvre au cours de ces travaux de thèse (les équipements « Hautes Pressions », leur mode d'utilisation, la préparation des cultures et des échantillons contaminés...).

Considérant l'ensemble des connaissances développées dans le domaine de l'application des HPH aux procédés de l'agro-alimentaire, différentes études ont été menées.

Le **Chapitre IV** concerne tout particulièrement l'action des (HPH) et l'échauffement du milieu au travers de la compression. Différents liquides alimentaires (lait écrémée, lait entier, jus d'orange) et différents milieux transmetteurs de la pression (eau, éthanol, éthylène glycol) ont été sélectionnés pour cette étude.

Le **Chapitre V** est consacré à l'inactivation de deux micro-organismes (*Escherichia Coli* et *Listeria innocua*) dans divers milieux alimentaires (lait jus d'ananas, jus de kiwi) en utilisant soit un traitement HPH continu soit un traitement HPH pulsé. D'autre part les effets induits par l'utilisation de dioxyde de carbone sous hautes pressions ont été également étudiés.

Ces divers travaux ont permis de mettre en évidence un certain nombre de résultats originaux.

- 1- Les effets thermiques liés à la compression doivent être pris en compte dans tout traitement de pasteurisation. La température initiale du produit et la cinétique de compression doivent être optimisées afin de pouvoir compenser l'accroissement de température.
- 2- La sensibilité des micro-organismes vis-à-vis de la pression est modifiée par les propriétés intrinsèques de l'aliment (pH, activité de l'eau, composition de la matrice alimentaire...). L'inactivation sous HPH des deux micro-organismes (*Escherichia coli* et *Listeria innocua*) dans le jus de kiwi était plus importante que celle observée au sein du jus d'ananas – milieu encore plus favorable à l'inactivation que le lait entier.

- 3- Les cinétiques d'inactivation sous HPH ne semblent pas suivre les cinétiques traditionnelles du 1^{er} ordre. Ainsi des modèles alternatifs ont du être étudiés pour ces cinétiques. Le modèle de Weibull peut être utilisé pour les cinétiques d'inactivation sous hautes pressions des micro-organismes.
- 4- Les traitements par pression pulsée constituent une alternative intéressante aux traitements conventionnels en continu. Cependant il est nécessaire d'optimiser divers paramètres : la durée de chaque pulse, le nombre de pulses et la valeur de la pression afin d'atteindre le niveau optimal de réduction des micro-organismes (*E-coli* et *L. innocua*), compatible avec une application industrielle.
- 5- La durée et la température du stockage des aliments après un traitement HPH doivent être optimisées afin d'accroître la sécurité des jus de fruits traités et d'éviter la reprise de croissance des micro-organismes endommagés.
- 6- Le traitement utilisant le dioxyde de carbone d'une part et la pression pulsée d'autre part peut être une voie efficace pour l'inactivation des micro-organismes dans le lait écrémé tout en réduisant le niveau de pression requis.

La principale conclusion de cette thèse est que les traitements sous Hautes Pressions Hydrostatiques (HPH) présentent un grand intérêt pour l'inactivation de micro-organismes dans des milieux divers (lait, jus de fruits) si les paramètres gouvernant le procédé (pression, température, durée, élévation de température liée à la compression, température initiale du milieu fluide transmetteur et de l'aliment, la nature de l'aliment et des micro-organismes contaminants) sont clairement identifiés et si le traitement (pression pulsée ou pression continue, utilisation de CO₂...) est adapté.

Ainsi les applications industrielles de tels traitements sous Hautes Pressions Hydrostatiques (HPH) de milieux alimentaires doivent prendre en compte divers facteurs d'ordre économique :

- (i) l'investissement de l'équipement Hautes Pressions qui est principalement lié au niveau de pression, à la valeur de la température (ambiante ou négative) et au mode d'application de la pression (continue ou pulsée),
- (ii) le coût de chaque traitement HPH prenant en compte notamment sa durée et la technique d'emballage du produit alimentaire.

Mots clés : Haute pression hydrostatique, chaleur de compression, cinétiques d'inactivation microbienne, *E. coli*, *L. innocua*, lait, jus de fruits, pression pulsée, haute pression de CO₂.

EXTENDED ABSTRACT

Consumers require foods of the highest quality in terms of natural flavour and taste, safety and without chemical additives.

The application of emerging technologies, such as high hydrostatic pressure, pulse electric fields, ultrasound and etc. can be utilized to replace, enhance or modify conventional techniques of food production. High hydrostatic pressure, as a non-thermal treatment, presents a lot of advantages. One of them is the result of two different factors: heat is not used and pressure -as a thermodynamical parameter- develops few energy. Consequently sensory and nutritional attributes (resulting mainly from molecules characterized by covalent bonds) remain unaffected, thus yielding products with better quality than those processed traditional methods.

Several publications have also demonstrated novel and diversified effects of this technology as the elimination of the allergenicity of food protein (Nogami et al. 2006) or the digestibility of high pressure treated starch with glucoamylases (Takahashi et al. 1994).

During the post two decades, this HHP technology has attracted a great research attention mainly related to:

- (i) the extension of keeping quality,
- (ii) the modification of the physical and functional properties of food systems,
- (iii) the use of the anomalous phase transitions of water under pressure, e.g. lowering the freezing point with increasing pressure due to the low compacity of the ice I. (common structural form at normal pressure).

The HHP technology in food processing can be summarized through mainly five key advantages:

- 1- the development of food processing at ambient temperature or even lower temperatures,
- 2- the instantaneous transmittance of pressure throughout the whole system whatever the size and the geometry of the sample,
- 3- the microbial death virtually eliminating heat damage and the use of chemical preservatives/additives, there by leading to improvements in the overall quality of foods,
- 4- the reduction – in some cases – of allergic character of foods or the improvement of the digestibility, leading to specific nutrition-applications,
- 5- the creation of novel foods with novel organoleptic and functional properties.

Chapter I is devoted to a brief History of the development of HHP in Biosciences and in Food Science in particular.

Chapter II summarizes the main effects of HHP on food components (including the micro-organisms).

Chapter III describes materials and methods (HHP equipments, HHP processing, preparation of bacterial species...) used for developing the research involved during this thesis.

Considering the above knowledge, different studies have been developed.

In **Chapter IV**: the action of high hydrostatic pressure (HHP) on compression (adiabatic) heating of selected liquid foods (skim milk, whole milk and orange juice) and pressure transmitting fluids (water, ethanol and ethylene glycol).

In **Chapter V** the inactivation of two microorganisms (*Escherichia coli* and *Listeria innocua*) in different food media (milk, pineapple and kiwifruit juices) by use of continuous or pulsed HHP and the effect of carbon dioxide during high pressure treatments was investigated.

These experimental results allowed pointing out some important results.

1. The thermal effects of compression should be taken into account when HHP pasteurization processes are developed. Initial temperature of the food product and compression rate should carefully be selected in order to compensate the compression heating.
2. The pressure sensitivity of microorganisms is affected by the general intrinsic food properties such as pH and water activity as well as the composition of the food matrix (inactivation of both microorganisms by HHP in kiwifruit juice was higher than the inactivation in pineapple juice which is higher than the inactivation in whole milk).
3. The HHP inactivation kinetics need not follow traditional first-order kinetics, hence alternative inactivation models are ought to be found. Weibull model can be used for HHP inactivation kinetics of microorganisms.
4. The pulsed pressure treatment could be an alternative to continuous HHP, but optimization should be done between the pulse holding time, the number of pulses and the pressure level to reach the desirable number of log-reduction of microorganisms (*E. coli* and *L. innocua*) compatible with an industrial application.
5. The storage duration and storage temperature after HHP treatment should carefully be optimized to increase the safety of HHP treated fruit juices since the growth of injured microorganisms can be avoided during storage.

6. The high pressure carbon dioxide (HPCD) treatment in combination with pulsed pressure can be an efficient way to inactivate the microorganisms in skim milk and to reduce the maximum pressure level for the desired log-reduction.

The main conclusion of this thesis indicated that HHP treatment has the potential to inactivate microorganisms in several foods (milk, pineapple and kiwifruit juices) if the process parameters (pressure, temperature, holding time, compression heating, initial temperature of the pressure transmitting fluid and food product, nature of the food and target microorganisms) are identified clearly and if the treatment (pulsed or continuous pressure treatment, HPCD) is selected accordingly.

In addition the industrial application of such HHP food processes must take into account different economical factors:

- (i) the investment of the high pressure equipment versus the pressure level, the initial temperature and the application of the pressure (pulsed or continuous),
- (ii) the cost of each HHP treatment including its duration, the food packaging...

Keywords: High hydrostatic pressure; compression heating; microbial inactivation kinetics;

E. coli; *L. innocua*; milk; fruit juice; pulsed pressure; high pressure carbon dioxide.

INTRODUCTION

Due to the researches involving the preservation of the organoleptic and the nutritional properties of foods, mild technologies for microbial stabilization are gaining more and more interest during these last twenty years. These researches have recently stimulated the development of non-thermal preservation techniques for food industry since temperature develops a lot of energy and consequently affects chemical bonds and structure.

Among these techniques, high hydrostatic pressure (HHP) has been investigated mainly during these last twenty years for manufacturing high quality food products and ingredients and has been intensively studied in different countries all over the world. Although large amounts of scientific works performed on the subject, relatively few commercial applications are reported due to the high fixed cost of HHP equipment and to the necessity of batchwise operation reducing the treated volume per day. Considering that the complexity and the cost of HHP equipment increase more than linearly with maximum operating pressure, the main requirement to make the HHP processes economically sustainable is the reduction of pressure level required to attain a commercially suitable microbial inactivation on the food processed without modifications of the organoleptic properties.

The main objective of this thesis was to use the high hydrostatic pressure (HHP) as the main parameter to investigate its effects in food science [compression heating of foods, inactivation of *Escherichia coli* and *Listeria innocua* in different food media (milk and fruit juices), pulsed pressure and high pressure carbon dioxide treatments].

CHAPTER I

BRIEF HISTORY OF THE DEVELOPMENT OF HIGH HYDROSTATIC PRESSURES IN BIOSCIENCES AND FOOD PROCESSING

I) High hydrostatic pressure (HHP) and the main factors characterizing such a thermodynamical parameter

I.1) Pressure and the earth

HHP is a characteristic parameter of the biosphere when it is viewed in terms of the volumes occupied by its major terrestrial (land) and aquatic components. Terrestrial habitats, where pressure value is close to one atmosphere (atm) [= 0.101 megapascal (MPa)] or lower, account for less than 1 % of the total volume of the biosphere. The oceans, which cover approximately 70 % of the earth's surface, have an average depth of 3800 m and consequently an average pressure of 381 atm (38.5 MPa). Approximately 79 % of the volume of the marine component of the biosphere lies below 1000 m. The greatest depth in the oceans, the "Challenger Deep" in the Marianas Trough, is near 11,000 m (Somero 1992).

The maximum pressure value at the center of the earth is about 3.7 Mbar [370 gigapascal (GPa)] and this value is higher for the giant planets. Approximately 90 % of the Universe is submitted to a pressure higher than 100 kbar (10 GPa). Consequently, pressure appears an important thermodynamical parameter at/or near the surface of the earth.

I.2) Pressure as a thermodynamical parameter

I.2.1) Types of pressure

Two types of pressure can be discussed:

- **Static pressures** where the pressure value can be maintained during a long time.

Among these static pressures two different categories can be defined:

- **Isostatic pressures** where the pressure value is same in all the directions of the space.

This is in particular the case in water (hydrostatic pressure).

• **Non-isostatic pressures** where a pressure gradient is induced versus the structure of the equipment generating the pressure or versus the non-homogeneous compressibility of the medium (in particular in the case of solids with an anisotropic structure).

- **Dynamic pressures** concern super-high pressures developed during a short-time and associated in the main cases with temperature. Shock-waves are mainly used for generating such pressures.

I.2.2) Definition of pressure

Pressure is defined as the force per unit area applied on a surface in a direction perpendicular to this surface. Mathematically: $P = F / A$,

where, P is the pressure, F is the normal force applied to the surface and A is the area of the surface. Megapascal: MPa [= 10^6 Pa], is the pressure unit commonly used in high pressure studies since the official pressure unit Pascal (Pa) corresponds to a very low pressure value ($1\text{Pa} = 1 \text{ Newton}/1\text{m}^2$) (*Newton represents a small force and 1 m^2 corresponding to a large surface*).

The conversion of MPa to other pressure units is given below:

1 MPa = 0.101 atm = 0.1 bar = 145.04 psi (pound per square inch).

I.3) Main factors characterizing pressure

Broadly three main factors can be discussed.

(i) Energy

When energy developed by high pressures is compared to the average value of the energy of chemical bonds, it can be underlined that energy developed by high pressure is quite low. Consequently high pressure would affect only weak chemical bonds. Table I.1 shows a comparison of the energy conveyed by pressure in different media (gas, liquid and solid) and the average energy of a chemical bond.

Table I.1. Energy developed by compression versus the nature of medium compared to the average energy of a chemical reaction (adapted from Demazeau et al., 2006)

Pressure (bar)	Medium	Energy (cal/mol)
1000	Gas	3000
1000	Solid	1
10,000	Solid	5
100,000	Iron	20
100,000	H ₂ O	1000
1	Chemical reaction	20,000

Table I.2 gives a comparison of the energy conveyed by pressure and temperature in the same medium (water). Such a comparison underlines that the energy developed by pressure is very small compared to that developed by temperature; consequently the phenomena induced by both parameters in biosciences will be completely different.

Table I.2. Increase of temperature and pressure of 1 liter of water with the same amount of energy

1 liter of H ₂ O		
Temperature (<i>T</i>)	<i>P</i> = 1 bar <i>T</i> = 20 → 25 °C	<i>E</i> ≈ 20 k joules
Pressure (<i>P</i>)	<i>T</i> = 20 °C <i>P</i> = 1 bar → 4000 bar	<i>E</i> ≈ 20 k joules

Pressure increases the dissociation of water and consequently k_e : [H⁺][OH⁻]. Such a phenomenon results from the electrostriction phenomenon (the positive or negative charges being rearranged in a more compact structure around the ions).

The dissociation $2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OH}^-$ is characterized by a negative ΔV value ($\Delta V = -22$ ml/mole of dissociated water) (Heremans et al. 1997), consequently the dissociation of water is improved under high pressure. At very high pressure conditions ($P \approx 5$ GPa) water can be considered as a melt-salt.

(ii) **Densification effect**

Under high pressures (due to compressibility) the difference between final and initial volumes (ΔV value) is always negative. This factor induces different phenomena as: (i) the formation of new structural forms of biological systems (such as different structural forms observed during the high pressure denaturation of proteins), or (ii) the dissociation of water (Heremans et al. 1997).

Taking into account these two factors (low conveyed energy and $\Delta V < 0$) characterizing pressure, as thermodynamical parameter, only the weak chemical bonds (characterized by a negative ΔV value) will be affected by the high pressure.

(iii) **The chemical reactivity.**

Temperature and pressure are used to shift reaction equilibria. The shift is determined by the change in the initial potential of the components involved. The pressure dependence of the chemical potential (μ_i) of a dissolved compound i is given in the following equation:

$$\left(\frac{\lambda \mu_i}{\lambda p} \right)_T = \bar{V}_i$$

Where \bar{V}_i is the partial molar volume of the compound i .

For a general chemical reaction at the equilibrium:

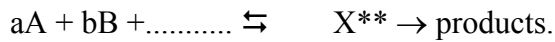


The equilibrium constant K_x as a function of the pressure is expressed in the equation below:

$$-RT \left(\frac{d \ln K_x}{dP} \right) = (l\bar{V}_L + m\bar{V}_M + \dots) - (a\bar{V}_A + b\bar{V}_B + \dots) = \Delta \bar{V}$$

$$\text{with } K_x = \frac{X_L^l X_M^m \dots}{X_A^a X_B^b \dots}$$

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:



$$K^{**}$$

Where X^{**} represents the transition state.

$$\text{Consequently } -RT \left(\frac{d \ln K_x}{dP} \right)_T = \Delta V^{**}$$

ΔV^{**} may be described as the sum of two components:

$$\Delta V^{**} = \Delta V^{**}_{\text{intrinsic}} + \Delta V^{**}_{\text{solv.}}$$

In reactions without important contributions of solvation effects $\Rightarrow \Delta V^{**} \approx \Delta V^{**}_{\text{intrinsic}}$.

Schematically bond formation, charge separation and concentration of equal charges result in volume contraction, bond scission, charge neutralization leading to volume expansion (Tauscher 1995, Van Eldik et al. 1989).

In Chemistry the application of high pressure leads to a strong development of the chemical reactivity inducing an improvement of the kinetics. Such a phenomenon has been – in particular – underlined in the investigations of the diffusion/impregnation of saccharose and NaCl under HHP (Lambert et al. 1997).

I.4) The development of high pressures in different scientific areas

The applications of high pressure in different scientific domains were strongly dependent on the development of the technologies which are able to generate such a parameter.

In the beginning high pressure was mainly applied in physics and chemistry. During the nineteenth century, pressures (in the range of few ten MPa to several hundred MPa) were developed for studying the compressibility of gases and liquids (Andrews 1861; Raoult 1890; Amagat 1893; Basset 1927; Bridgman 1949). High pressure was used in chemistry for reproducing the synthesis of minerals (mainly as single crystals – in particular α -quartz) but the main success was the synthesis of diamond at the middle of the twenty century either A.S.E.A. (Sweden) [Liander et al. 1961] or G.E. (US) [Bovenkerk et al. 1959].

The first industrial application of high pressure was the synthesis of NH_3 at the beginning of the 20th century (Travis 1998), then different processes were developed in material chemistry (diamond for developing machining or cutting processes for super-hard alloys (Sung 2001), CrO_2 for magnetic recording applications (Demazeau et al. 1980).

I.5) Development of high pressures in biosciences

High pressure treatment to kill bacteria was first described in 1895 by Royer. Hite et al. (1899) have studied the preservation of milk under high pressure. Bridgman (1914) investigated the coagulation of albumen under pressure but such a phenomenon was elucidated by Grant et al. (1941) as the protein denaturation.

Hite et al. (1914) developed the hydrostatic pressure to inactivate certain microorganisms in order to preserve fruits and vegetables. Between 1927 and 1952, James and Jacques Basset and their coworkers developed high pressure in order to inactivate different micro-organisms for either food processing development or medical applications

(Basset, 1927; Basset and Macheboeuf 1932; 1933; Basset et al. 1933; 1935a; 1935b; Macheboeuf and Basset 1936; Barbu et al. 1948; Dubert et al. 1952). Effect of HHP on marine bacteria was also investigated (Zobell and Johnson 1949; Zobell and Cobet 1962; Zobell 1964; Zobell 1970).

Such research activities were maintained by different scientific groups during the sixties and the seventies. It is possible in particular to mention: Timson and Short's (1965) research works on pressure effects on microorganisms in raw milk, Gould and Sale's (1970) research work involving the germination of spores by hydrostatic pressure, Wilson's (1974) works on the sterilization of low acidic foods using pressure and pasteurization temperatures, Elgasim and Kennik's (1980) research activities concerning the pressure effects on beef protein. Charm et al. (1977) suggested the use of pressure for long-term refrigerated storage of foods. Marquis (1976), Marquis and Matsumura (1978) studied the response of biological systems to hydrostatic pressure up to 110 MPa.

During the eighties and the beginning of nineties, researches involving HHP and biosciences were increased: high pressure effects on enzymes (Morild 1981), high pressure effects on proteins and other biomolecules (Heremans 1982), the biological effects of high hydrostatic pressure on food microorganisms (Hoover et al. 1989), applications high pressure homogenization for food preservation (Popper and Knorr 1990), the high pressure technology in food industry (Farr 1990), the role of high pressures on the living systems (Regnard 1994a; 1994b) were investigated.

Strong efforts for setting up new food processes treated by HHP were developed in particular in Japan (Hayashi 1989; 1990; Horie et al. 1991; Ogawa et al. 1990; Tanaka and Hatanaka 1992). These developments in Japan can be explained by different factors: (i) the difficulty to use ionizing treatments (ex: radiation), (ii) the preservation of the organoleptic properties of the raw material (the Japanese cooking culture), (iii) the growth of novel

processes supported by a novel technique. Such research activity has led to induce: (i) a strong interest, in basic research, to explain the mechanism of microorganisms inactivation (Balny et al. 1992; Masson 1992; Knorr 1993; Hoover 1993; Tonello 1993; Demazeau 1993; Cheftel 1995; 1997; Tauscher 1995), (ii) new industrial processes for food preservation (the first food product stabilized under high pressures was marketed in Japan at the beginning of the nineties).

During then last fifteen years HHP technology in food processing has steadily increased. Several products are now on the market in different countries: fruit juices, jam, toffu, ham, shellfish and bio-polymers (such as proteins or starches). At the present time approximately 90 industrial equipment installations are in operation in the world. The volumes of the HHP equipment vary from 35 to 360 L. (Tonello-Samson 2007).

The major task of HHP applications in food industry is the extension of shelf-life or the elimination of microbial pathogens. The viability of vegetative microorganisms is affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems responsible for the control of metabolic reactions (San-Martin et al. 2002; Matser et al. 2004; Knorr et al. 2006; Rastogi et al. 2007). In addition, different physico-chemical parameters were added to HHP treatments: low or sub-zero temperatures (Kalichevsky et al. 1995 ; Le Bail et al. 2002 ; Urrutia-Benet et al. 2004 ; Luscher et al. 2004; Dumay et al. 2006; the use of carbon dioxide (Parton et al. 2007 (a); Parton et al. 2007 (b); Garcia-Gonzalez et al. (2007). In parallel during these last years HHP were also investigated for biotechnological applications (Balny et al. 1992; Mozhaev et al. 1994; Rigaldie et al. 2001; Rigaldie and Demazeau 2004).

General studies involving the development of pressure indicators for HHP processing of foods (Minerich and Labuza 2003); food safety (Fonberg-Broczek et al. 2005) or “commercial opportunities and research challenges in high pressure processing of foods” (Torres and Velazquez 2005) underline the strong interest to HHP applications.

CHAPTER II

EFFECTS OF HIGH HYDROSTATIC PRESSURE ON FOOD CONSTITUENTS

The different constituents of foods are: water, carbohydrates, proteins and enzymes, lipids, vitamins and flavors.

II.1) Water under high pressure

Water is the main component of the foods and is frequently used as a pressure transmitting fluid due to safety and economical reasons. The volume decrease for water is approximately 4 % at 100 MPa, 7 % at 200 MPa, 11.5 % at 400 MPa, 15 % at 600 MPa at 22 °C. Moreover, compression values of water can reach to 30 % for pressures around 900 MPa.

II.1.1) The phase diagram of water versus pressure and temperature

The phase diagram of water under pressure, which was introduced by Bridgman in 1912, is given in Figure II.1. Under high pressure, water shows an unusual freezing point depression to -22 °C at 210 MPa. Furthermore, water remains liquid for example, at -10 °C under the pressures of 200, 250, 300, 350 and 400 MPa, being its point of fusion lowered by the pressure.

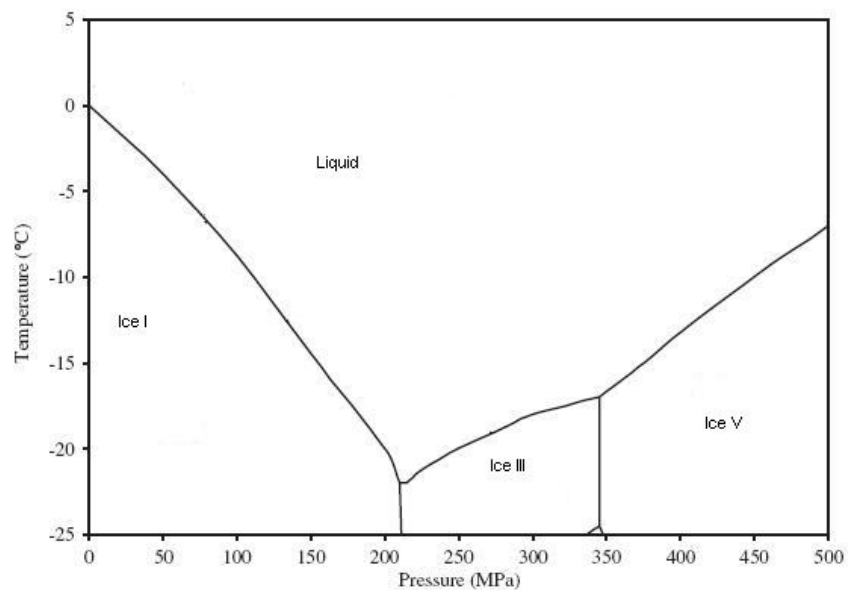


Fig. II.1. Phase diagram of water under high pressure (adapted from Özmütlu et al. 2006). The phase transition lines are based on the data reported by Brigman (1912).

At subzero temperatures, in relation to the phase diagram of water, interesting applications such as pressure assisted freezing or subzero cooling without ice formation can be induced (Picart et al. 2004).

II.1.2) Adiabatic heating (Compression heating)

The treatment of foods or food ingredients by using HHP is accompanied by an increase of the temperature in the treated volume. This phenomenon is induced by compressive work against intermolecular forces. The improvement of temperature correlated to compression can be theoretically calculated by using Eq. (2.1) (Denys et al. 2000).

$$\frac{dT}{dP} = \frac{T \cdot \alpha}{\rho \cdot C_p} \quad (2.1)$$

where T is temperature (K), P is pressure (Pa), ρ is density ($\text{kg} \cdot \text{m}^{-3}$), C_p is heat capacity of the food substance at a constant pressure ($\text{J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$) and α is coefficient of thermal expansion (K^{-1}). This equation is strictly applicable to only small pressure changes. The compression rate should be very low (less than $0.1 \text{ MPa} \cdot \text{s}^{-1}$). This condition would impose a time longer than 1 h to reach 400 MPa which is not convenient for food industry (Otero and Sanz 2003). Therefore it is almost impossible to avoid compression heating during HHP treatment whatever the structure of the equipment is.

The adiabatic compression of water causes an increase of the temperature of 2-3 °C per 100 MPa (it depends on the initial temperature and the rate of compression). The decompression causes a cooling of the same order of greatness.

II.2) Effects of HHP on carbohydrates

HHP does not affect simple sugars such as monosaccharides and oligosaccharides since they are constituted by covalent bonds; however, polisaccharides constituted by chains of monosaccharides from of the weak bonds can be affected by HHP. For example starch

structure could be damaged by HHP; it causes gelatinization similar to the gelatinization obtained by thermal treatment. The behavior of the starch under pressure is varying according to its origin, the composition of the medium to pressurize, the intensity of the pressure, the temperature and duration of the pressurization (Kawai et al. 2007).

II.3) Effects of HHP on proteins and enzymes

HHP can denature protein molecules (Fig. II.2). The four levels of protein structure are characterized as: primary (amino acids in a polypeptide chain joined by covalent bonding), secondary (coiling of peptide chains joined with hydrogen bonding), tertiary (arrangement of chains into globular shape by non-covalent bonding) and quaternary (various compact structures or sub-units joined by non-covalent bonding). Pressure denaturation of proteins is a complex phenomenon depending on the protein structure, pressure range, temperature, pH and solvent composition. The secondary, tertiary and quaternary structures can be significantly affected by HHP (because high pressure affects non-covalent weak bonds); therefore HHP may result in novel functional properties because tertiary structure is important in determining protein functionality. The main targets of pressure are the electrostatic and hydrophobic bonds in protein molecules. Protein denaturation becomes irreversible beyond a given pressure threshold which depends on the protein.

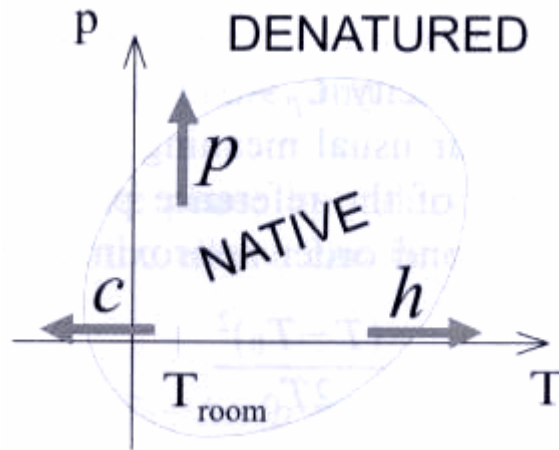


Fig. II.2. Schematic representation of the elliptic phase diagram of proteins. The arrows marked by the letters p, h, c show the specific denaturation ways known as pressure, heat and cold denaturation. (adapted from Smeller 2002)

HHP affects enzymes in a variety of ways depending on other parameters of processing and also the type of the enzyme. Exposure to high pressure may activate or inactivate enzymes versus the ΔV value. Pressure inactivation of enzymes is influenced by the pH, substrate concentration, subunit structure of enzyme and temperature during pressurization. Pressure effects on enzyme activity are expected to occur at the substrate-enzyme interaction. If the substrate is a macromolecule, then the effects may occur on the conformation of the macromolecule, which can make the enzyme action easier or more difficult. Pressure enzyme inactivation can also be attributed to alteration of intermolecular structures or conformational changes at the active side. Inactivation of some enzymes pressurized to 100-300 MPa is reversible. Reactivation after decompression depends on the degree of distortion of the molecule. The changes of reactivation decrease with an increase in pressure beyond 300 MPa (Morild 1981; Gross and Jaenicke 1994; Buzrul 2003).

II.4) Effects of HHP on vitamins and flavors

Vitamins are small molecules; their structures are composed of covalent bonds, so they are not very sensitive to HHP. Some vitamins of chicken egg, as vitamins A, E, B2 and the folic acid, do not show any molecular alteration or denaturation after pressure treatment (Hayashi 1989). In case of flavor compounds (aldehydes, esters...) the covalent bonds are also numerous and prevalent, so this can explain their barotolerance and also the preservation of the tastes and flavours of food product pressure treated (Cheftel 1995).

II.5) Effects of HHP on lipids

The primary effects of pressure on phospholipids can be observed on the temperatures of the transitions. Pressure favors the crystalline state, as a result of the Le Chatelier principle (Heremans, 1995). As pressure increases at constant temperature, the lipid bilayer adapts by changing its conformation. A variety of pressure-induced phase transformations has been observed such as liquid-to-gel transition and gel-to-interdigitated gel transition. In contrast to Le Chatelier's principle, the volume of the lipid bilayer in liquid- to- gel transition increases with an increase in the fatty acyl chains length of the phospholipids. Pressures of 50-200 MPa cause the transition from the liquid-crystalline to gel. At higher pressures (above 200 MPa), a second pressure-induced phase transition, called interdigitated phase, is observed, and the bilayer volume decreases by 5%, accompanied by a decrease in its thickness (Kato and Hayashi, 1999; Winter, 2002).

II.6) Effects of HHP on sensory and nutritional characteristics

The principal advantage of high pressure technology is its relatively small effect on food composition and hence, on sensory and nutritional attributes. For instance; grapefruit juice manufactured by high pressure technology does not possess the bitter taste of limonene

present in conventional thermal processed grape fruit juice (Buzrul 2003). The jams obtained by high pressure processing retain the taste and color of fresh fruit, unlike conventional jams produced by heat (Dervisi 2001).

Protein, nucleic acid and starches, whose tertiary structures are composed of non-covalent bonds, change their structures at high pressure and lead to denaturation, coagulation or gelatinization. Pressure effects are, thus, similar to heat effects on biological materials and foods. In other words, high pressure is as useful as high temperature (Table II.1).

Table II.1. Possible uses of high pressure in processing and preservation of foods as compared to high temperature processing. (Adapted from Hayashi, 1995)

Phenomenon	Temperature	Pressure
Sterilization of microorganisms	√	√
Killing of insects and parasites	√	√
Denaturation of protein	√	√
Coagulation of protein	√	√
Gelatinization of starch	√	√
Chemical changes*	√	X
Enzyme inactivation	√	√

√ : possible, X : not possible, *e. g. Maillard reactions, off-flavor and vitamin destruction

The unique advantage of the HHP treatment is that the covalent bonds are not affected as the liquid water was compressed. A wide variety of effects and changes in food flavor, texture, physical appearance and structure could result after the application of pressure and these changes depend on the type of food and its composition and structure. However, effects such as Maillard reaction and formation of cooked flavors do not occur during the pressure treatment. Thus, it is possible to retain natural flavor and taste by application of high pressure treatment to foods (Hayashi 1995).

II.7) Effect of HHP on microorganisms

II.7.1) Influence of HHP on the different microorganisms

Food preservation is based primarily on the inactivation, growth delay or prevention of spoilage and pathogenic microorganisms. A major function of high pressure processing of food is destruction of microorganisms. The effectiveness of HHP on microbial inactivation has to be studied in great detail to ensure the safety of food treated in this manner (Buzrul 2003).

It can be expected that the mode of action of pressure on whole organisms is not necessary the same, but dependent on the pressure level. Elevated hydrostatic pressures between 30 and 50 MPa can influence gene expression and protein synthesis. At pressures of about 100 MPa, the nuclear membrane of yeasts was affected, and at more than 400-600 MPa further alteration occurred in the mitochondria and the cytoplasm. In particular, metal ions are released at pressures over 300 MPa. Pressure-inactivation is also accompanied by an increase of extracellular ATP showing leakage of the membrane (Smelt 1998).

The result is an increase in the permeability of cell membranes and possible inhibition of enzymes vital for survival and reproduction of the bacterial cells. Exponentially growing cells are more sensitive to pressure than cells in the stationary phase. Stress might be induced during the stationary phase (e.g. through starvation or acidification). In general, Gram-negative bacteria are inactivated at a lower pressure than Gram-positive bacteria (Hoover 1993; Knorr 1993; Alpas et al. 1999; Alpas and Bozoglu 2000). The lower resistance of Gram-negative bacteria compared with Gram-positives has attributed to their lack of teichoic acid, which is responsible for the rigidity of the cell wall of Gram-positive bacteria (Hoover 1993; Alpas et al. 2000). Microbial cells surviving pressurization also become sublethally

injured and developed sensitivity to physical and chemical environments to which normal cells are resistant (Alpas 2000).

In general, bacterial spores are more resistant to HHP than vegetative cells (Lechowich 1993). This has been attributed to the protection afforded by dipicolinic acid (DPA) of the spore proteins against solvation and excessive ionization that are responsible for cell death (Alpas 2000). Bacterial spores are killed directly by pressures higher than 1000 MPa. However, spores are sensitive to pressures between 50 and 300 MPa since low pressure values can cause spore germination. Release of DPA, which is not present in vegetative bacteria, is one of the first events in germination. *Bacillus subtilis* in physiological saline loses 80 % of its DPA after 60 MPa at 30 °C. At pressures over 1000 MPa, spores are killed more rapidly at low pH values (Smelt 1998).

Viruses have a high degree of structural diversity, which results in a wide range of pressure resistances. There are two potential applications of pressure inactivation of viruses: vaccine development and virus sterilization. Pressurization inactivates viruses but preserves their immunogenic properties, because usually it does not markedly change viral structure (Mor-Mur and Yuste 2005). The most common human enteric viruses are Norwalk-like viruses, hepatitis A, rotavirus and human astrovirus. Complete inactivation of suspensions of feline calicivirus (a Norwalk-like virus surrogate), adenovirus and hepatitis A can be achieved by treatment at 275 MPa for 5 min, 400 MPa for 15 min and at 450 MPa for 5 min, respectively (Hogan et al. 2005).

II.7.2) Physiological response of microorganisms during HHP inactivation

Microbial inactivation caused by thermal or non-thermal inactivation techniques can exhibit different kinds of inactivation patterns. Fig. II.3 shows several characteristic inactivation curves is shown such as biphasic, concave upward, biphasic with a shoulder and concave downward curves.

In the case of different inactivation processes, different types of microbial inactivation may be observed depending on the strain as well as on the composite of the food matrix. For the effective use of HHP processing in the food industry, it is of special interest to detect process parameter relations resulting in decreased microbial risk over a period of time. Therefore, all modifications of parameters (microbial strain, food compounds, process temperature and pressure) have to be analysed in terms of either successful or ineffective inactivation of microorganisms. This increased knowledge base concerning the various effects of process parameters (pressure, pressure holding time, temperature, additives, etc.) is a prerequisite for predictive models of bacterial inactivation during the HHP process and its application in the food industry (Kilimann 2005).

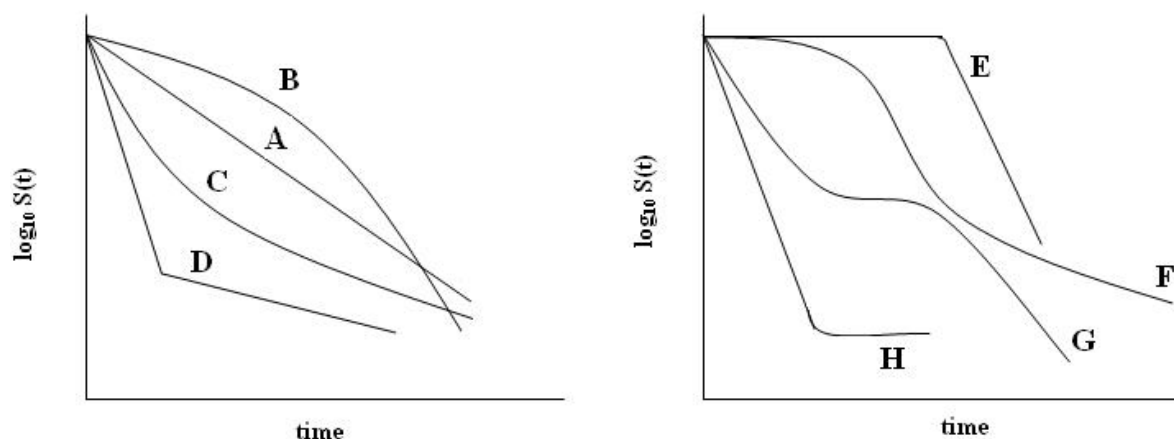


Fig. II.3. Commonly observed types of survival curves. Left plot: Linear (A), Downward concave (B), Upward concave (C) and biphasic (D). Right plot: Linear with a preceding shoulder (E), Sigmoidal I – starting with a downward concavity and ending up with an upward concavity (F), Sigmoidal II – starting with an upward concavity and ending up with a downward concavity (G), Linear with tailing (H). Adapted from Xiong et al. (1999), Peleg (2003) and Geeraerd et al. (2005).

II.7.3) Extrinsic factors affecting the HHP sensitivity of microorganisms

HHP is no different from other physical preservation methods in that its effectiveness against microorganisms is influenced by a number of different factors. These all interact and contribute to the lethal effect and therefore have to be considered when designing process conditions to ensure the microbiological safety and quality of pressure-treated foods (Patterson 2005).

II.7.3.1) Nature of the food substance

The *chemical composition* of the food substance during treatment can have a significant effect on the response of microorganisms to pressure. Certain food constituents such as proteins, carbohydrates and lipids can have a protective effect (Simpson and Gilmour 1997). Inactivation data obtained using buffers or laboratory media, therefore, should not be extrapolated to real food situations where a more severe pressure treatment may be needed to achieve the same level of inactivation. For example, a treatment of 375 MPa for 30 min at 20 °C in phosphate buffer (pH 7.0) gave a 6 log₁₀ inactivation of a pressure-resistant strain of *Escherichia coli* O157:H7. However, the same treatment gave a 2.5 log₁₀ reduction in poultry meat and only 1.75 log₁₀ reduction in milk (Patterson et al. 1995). Cations, such as Ca²⁺, can be baroprotective and this may explain why many microorganisms appear more pressure resistant when treated in certain foods, such as milk (Hauben et al. 1998). A low water activity (a_w) protects microorganisms against the effects of pressure (Palou et al. 1997). Oxen and Knorr (1993) reported that reducing a_w of the medium from 0.98-1.0 down to 0.94-0.96 resulted in better survival of *Rhodotorula rubra* when it was subjected up to 200-400 MPa for 15 min at 25 °C.

The *pH* of acidic solutions decreases as pressure increases and it has been estimated that in apple juice, there is a pH drop of 0.2 U per 100 MPa (Heremans 1995). To date, the pH

change which occurs during pressure treatment cannot be measured directly in solid food, but methods have been developed for *in situ* pH measurement during pressure treatment of liquids (Hayert et al. 1999; Stippl et al. 2004). When the pressure is released, the pH reverts to its original value but it is not known whether these sudden changes in pH affect microbial survival in addition to the effect of pressure. It is known that pH and pressure can act synergistically leading to increased microbial inactivation. Linton et al. (1999) has shown that initial pH had a significant effect on inactivation rates of *E. coli* O157:H7 in orange juice. As pH was lowered, the cells were more susceptible to pressure inactivation and sublethally injured cells failed to repair and died more rapidly during subsequent storage of the juice.

Food additives can have varying effects on microbial resistance to pressure. Pressure has been used to sensitize Gram-negative bacteria such as *Salmonella*, as well as Gram-positive bacteria such as *Listeria monocytogenes* to nisin and lysozyme (Kalchayanand et al. 1998; Masschalk et al. 2001). The combination of high pressure and nisin was also used to increase the inactivation of *Bacillus cereus* spores in cheese (López-Pedemonte et al. 2003). However, in this case the pressure treatment conditions were relatively severe. The treatment (60 MPa at 30 °C for 210 min to germinate the spores followed by 400 MPa at 30 °C for 15 min to kill the vegetative cells) was carried out in the presence of 1.56 mg.l⁻¹ nisin in the cheese. This resulted in approximately 2.4 log₁₀ inactivation of the spores. Pediocin AcH also works synergistically with pressure. A combination of 345 MPa for 5 min at 50 °C in the presence of 3000 AU ml⁻¹ pediocin AcH gave at least a 7 log₁₀ inactivation of a range of bacteria including *L. monocytogenes*, *S. Typhimurium*, *Staphylococcus aureus*, *E. coli* O157:H7, *Lactobacillus sake* and *Pseudomonas fluorescens*. This level of inactivation could not be achieved by pressure alone (Kalchayanand et al. 1998). Similarly, the combination of high pressure with other antimicrobial agents such as lacticin 3147 (Ross et al. 2000),

lactoperoxidase (Garcia-Graellis et al. 2003) and carvacrol (Karatzas et al. 2001) can work synergistically to enhance the kill of microorganisms, including pathogens.

II.7.3.2) Effect of temperature

Temperature during pressure treatment can have a significant effect on microbial survival. Increased inactivation is usually observed at temperatures above or below 20 °C (Takahashi et al. 1992). High temperatures (> 70 °C) can be particularly effective in helping to achieve high pressure sterilization. The combination of elevated temperatures (< 50 °C) with pressure has also been suggested as a practical way to overcome the problem of pressure resistant strains of vegetative cells. Patterson and Kilpatrick (1998) reported approximately 6 log₁₀ inactivation of a pressure-resistant *E. coli* O157:H7 in poultry mince and 5 log₁₀ inactivation in milk using a treatment of 400 MPa at 50 °C for 15 min. Neither heat nor pressure alone could achieve this level of inactivation. Refrigeration temperatures can also enhance pressure inactivation. Gervilla et al. (1997) reported that ewe's milk pressurized at 450 MPa at 2 °C for 15 min was more effective at inactivating *L. innocua* than the same treatment at 25 °C but less effective than the pressure treatment at 50 °C. Nevertheless, temperature during HHP treatment should be limited in order to avoid the adverse effects of temperature on food flavor.

II.8) Impact of HHP treatments on the improvement of microbiological quality of foods

HHP-treated fruit jams and sauces first became commercially available in Japan in the early 1990s. Treatment of fruit jams with around 400 MPa for up to 5 min at room temperature can significantly reduce the number of microorganisms, especially yeasts and moulds. Refrigeration of the jam after processing is necessary due to browning and flavour changes caused by enzymatic activities. These products have a shelf-life of around 30 days

and have superior sensory quality compared with those prepared in a conventional manner (Ludikhuyze and Hendrickx 2001).

Fruit juices are normally processed at 400 MPa or greater for a few minutes at 20 °C or less. This can significantly reduce numbers of yeasts and moulds and so extend shelflife for up to 30 days. Pathogens, such as *E. coli* O157:H7 can also be destroyed by this treatment (Linton et al. 1999; Ramaswamy et al. 2003). Pressure-treated orange and grapefruit juices have been available in France since 1994, while pressure-treated apple juice is available in Italy. HHP treatment of vegetable products is problematic because of their relatively high pH along with the possibility of survival and growth of pathogenic spore-forming organisms. However, one of the most successful pressure-treated foods in the USA is guacamole. Its market share continues to grow and is reportedly based on the consumer preference for the “fresher” taste of guacamole processed in this manner compared with heat-treated or frozen products. Treatment of around 500 MPa for 2 min is sufficient to extend shelflife from 7 to 30 days at refrigeration temperatures. Challenge studies with a variety of pathogens have shown that this treatment is sufficient to give a 5 log₁₀ reduction in numbers (Parnell 2003) and the process has been approved by the US Food and Drug Administration. Sliced cooked ham and other delicatessen meat products, in flexible pouches, can be successfully treated using 500 MPa for a few minutes. The sensory properties of ham are preserved, and shelf-life can be extended to 60 days under chilled storage. Cooked delicatessen products have a risk of post processing contamination from pathogens such as *L. monocytogenes*. High pressure treatment as a final preservation step, after packaging, can give additional microbiological safety assurance. During challenge studies a treatment of 500 MPa can cause a 5 log₁₀ reduction in *L. monocytogenes* in dry-cured ham (Minerich and Krug 2003). HHP-treated cooked and vacuum-packaged ham is available in Spain and in the USA. Another example of commercially successful pressure treated foods available in the USA is oysters. The initial

aim of the pressure treatment was to eliminate *Vibrio* spp. from oysters, which are often eaten raw or only lightly cooked. *Vibrio* spp. are relatively sensitive to high pressure, although there can be species variation (Cook 2003). Typical treatments of 250-350 MPa for 1-3 min at ambient temperature are used commercially without significantly affecting sensory quality. An additional benefit of pressure-treated oysters is the mechanical shucking effect it causes, releasing the adductor muscle from the shell (He et al. 2002). For this reason, a heat shrink plastic band is placed around each oyster prior to processing so that the shell is kept shut until the meat is required. The main processor of pressure-treated oysters in the USA uses a trademark plastic gold band and these products have achieved several national awards for quality products. Pressure will successfully shuck other shellfish, such as mussels, Nephrops and crabs as well as improving their microbiological quality and it is likely that the technology will be more widely used for this purpose. There is also increasing interest in pressure-treating fin fish to improve microbiological safety and quality as well as using the technology to produce a range of novel food products (Lakshmanan and Dalgaard 2004), including surimi gels (Ohshima et al. 1993; Ashie and Simpson 1996).

Pressure treatment of milk and dairy products to improve microbial safety and quality has been of interest since the early work of Hite (1899). However, it is likely that the technology will only be used commercially for niche applications, where it can provide a commercial advantage over existing, usually heat-treated, products. For example, pressure treatment may be of value in treating milk that is to be used in the manufacture of raw milk cheese, where it could reduce the numbers of pathogens such as *L. monocytogenes*. However, some pathogens, such as certain strains of *E. coli* O157:H7, are known to be extremely pressure resistant in milk (Patterson et al. 1995). Therefore, this approach will not solve all the microbiological safety problems associated with raw milk cheese. High pressure treatment of yoghurt has also been investigated. Tanaka and Hatanaka (1992) investigated the

effectiveness of using high pressure to prevent after-acidification of yoghurt during storage. They concluded that a treatment of 200-300 MPa for 10 min at room temperature prevented the continued growth of lactic acid bacteria during storage and so maintained the yoghurt quality.

The range of products now being considered for high pressure treatment continues to grow year-on-year. A range of complete meal kits have recently been launched in the USA. The kits consist of pressure-treated cooked meat or chicken, salsa, guacamole, peppers and onion are now also available. Only the flour tortillas are not pressure treated. The products have a chilled shelf-life of at least 30 days and only required to be reheated in a microwave before consumption. It is likely that the range of added-value, high quality pressure-treated foods will increase within the next 5 years.

II.9) Objectives of the research under HHP conditions

The objectives of this thesis were:

1. To study the compression heating effect of different liquid foods and pressure transmitting fluids during HHP treatment.
2. To investigate the pressure inactivation kinetics of microorganisms in foods.
3. To reduce the maximum pressure level of HHP processes for the microorganisms inactivation by use of pulsed pressure treatments.
4. To reduce the maximum pressure level of HHP processes by use of carbon dioxide as an additive.

CHAPTER III

MATERIALS AND METHODS

III.1) Description of the HHP equipment

Pressurization of samples was carried out using a HHP computer controlled equipment. This equipment designed by NFM–Technologies (Le Creusot, France) and FRAMATOME (Paris, France), marketed by CLEXTRAL (Firminy, France). The schematic drawing of this equipment is represented in Fig. III.1. The corresponding photography of this equipment is given in Fig. III.2.

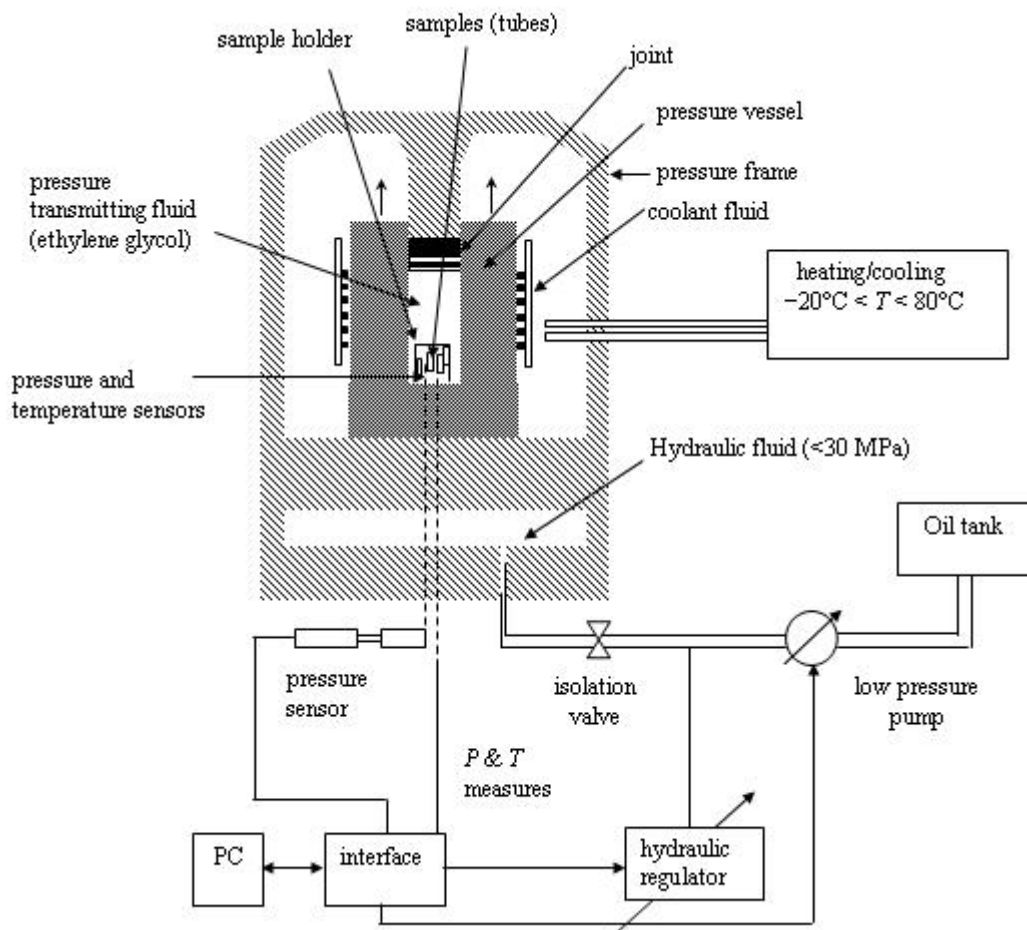


Fig. III.1. Schematic drawing of HHP equipment



Fig. III.2. HHP equipment used in this study

The main characteristics of the equipment are:

- (i) A high pressure vessel with a volume of 3 L (diameter: ~ 10 cm, height: ~ 40 cm), this volume is adapted either for basic researches or pilot-plant experiments,
- (ii) Maximum pressure value close to 800 MPa.
- (iii) Pressure is increased through the displacement of the vessel along the piston, this displacement is induced by an actuator using oil at medium pressure ($P = 20\text{-}30$ MPa) (Fig. III.1).

The food samples are isolated by sealed bags. The composition of the package is dependent of the experimental conditions (pressure and temperature). Previous studies had allowed selecting the most appropriated packaging among the available food packages on the market (Demazeau et al. 2008).

The pressure transmitting fluid was selected as ethylene glycol due to: (i) its low freezing temperature (e.g. at $-20\text{ }^{\circ}\text{C}$ and 0.1 MPa) allowing HHP experiments at subzero temperature, (ii) its higher density (than water) able to prevent any leakage of the liquid from the pressure vessel.

Although the maximum compression and release rate was $375\text{ MPa}\cdot\text{min}^{-1}$, the come-up and pressure release time was set to $300\text{ MPa}\cdot\text{min}^{-1}$ due to safety considerations. The samples were put into a sample holder and the sample holder was placed into the pressure vessel. Pressure and temperature were measured using sensors inside (at only one location close to the samples) and outside the high pressure vessel and all data were stored in the computer system. The regulation of the temperature of the pressure transmitting fluid into the HHP vessel is managed by a circulating fluid between the two envelopes of the HHP equipment (the external wall of the HHP vessel and the external envelop) (Fig. 4). The maximum interval between measurements was 0.5 s . The error introduced by the recording system was considered not significant.

All data (pressure of the oil used for the actuator, temperature and pressure of the fluid transmitting medium versus time etc...) are recorded in order to manage the computer system used for programming the HHP experiments. This computer system is represented in Fig. III.3.

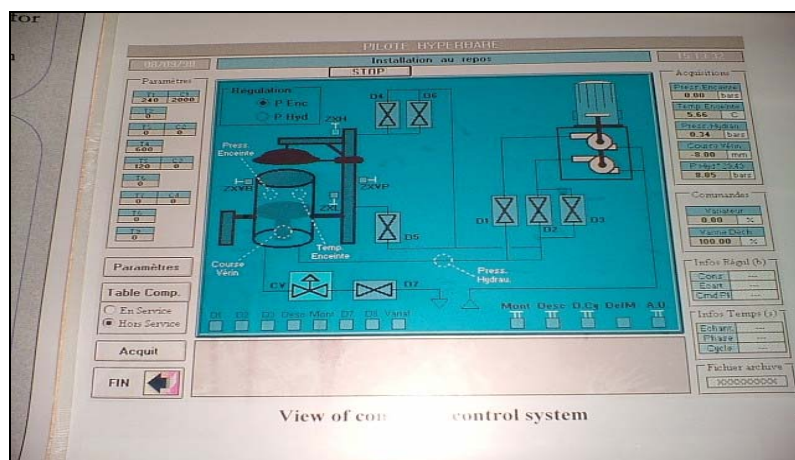


Fig. III.3. Computer control system of the HHP equipment.

III.2) Preparation of bacterial species

The microorganisms used were *E. coli* ATCC 11775 and *L. innocua* ATCC 33090 (both from E.R.A.P. laboratory – Périgueux, France). Previous studies indicate both *E. coli* ATCC 11775 (Garcia-Graells et al. 2000) and *L. innocua* ATCC 33090 (Tay et al. 2003) are pressure-resistant microorganisms. The strains were maintained on tryptic soy agar plus 0.6 % yeast extract (TSAYE) (Merck, Darmstadt, Germany) slants. For growth, a loopful of each organism was transferred to tubes of tryptic soy broth supplemented with 0.6 % yeast extract (TSBYE) (Merck, Darmstadt, Germany) at 37 °C for 15-21 h and transferred to fresh broth every 48 h for use in this study.

Food samples [Ultrahigh-temperature (UHT) skim and whole milk (pH 6.64), kiwifruit (pH 3.32) and pineapple juices (pH 3.77)] were inoculated with pure cultures from the early stationary phase to obtain about 10^7 to 10^9 colony forming units (CFU) mL⁻¹ food sample. All samples were inoculated at least 2 h before treatment to allow cells to adapt to the new environment.

III.3) Different experiments managed under HHP

III.3.1) Compression Heating Experiments

III.3.1.1) Pressure transmitting fluids and liquid foods

Water, ethanol (99.5%, J.T. Baker, Deventer, Holland), and ethylene glycol (99%, Sigma Aldrich, Steinheim, Germany) were used as pressure transmitting fluids. Whole milk, skim milk, and orange juice were obtained from a local market (Bordeaux, France).

III.3.1.2) Parameters

Pressurization was carried out at 100, 200, 300 and 400 MPa. Ideally, pressures should not be greater than 350 MPa, to reduce capital investment costs of HHP processes, although successful commercial applications have used higher pressures than this (Jordan et al. 2001). That is why the maximum pressure has been selected as 400 MPa in this study.

The compression rates were 100, 200, and 300 MPa/min. These values were also applicable to commercial large-scale applications. The decompression rate was set to its fastest value (300 MPa/min), as most commercially available HHP systems had fast decompression rates. Prior to pressurization, the sample was introduced directly into the high pressure vessel (3 L) and by using a regulating device the initial temperature (5, 20, and 35 °C) was set (Fig. 4). The vessel was thermoregulated by means of a laterally surrounding isolated coil and ethylene glycol was used as the thermoregulating fluid to keep the temperature of the pressure transmitting fluid constant. The equipment operates with a piston connected to a pump (direct compression system), which allows pressurization of the liquid foods without packaging.

When all the initial conditions were obtained, the air inside the vessel was removed, the high pressure system was initiated, and the set pressure was reached. The process time was one minute and did not include the come-up or depressurization time.

The compression heating value was calculated by taking the difference between the initial temperature and the maximum compression temperature of the sample at the target pressure. Although the temperature was measured at one location within the chamber, temperature varied both temporally and spatially during a pressure cycle (Carroll et al. 2003); however, the experience with the system used indicated that the temperature gradients vanished quickly and temperature uniformity within the chamber was accomplished. Under

large scale conditions, the variations of the temperature in the vessel would probably be less (Ting et al. 2002). All the experiments were repeated three times.

III.3.2) Microbial inactivation experiments

III.3.2.1) Microbial inactivation experiments in whole milk

The microbial strains in whole milk were pressurized in duplicate at 400, 450, 500, 550 and 600 MPa for various times at about 22 °C, since potential industrial applications of HHP in milk (without additives and at room temperature) is more likely to be in the range of 400-600 MPa (Chen and Hoover 2004).

III.3.2.2) Pulsed HHP treatments for the microbial inactivation in whole milk

E. coli and *L. innocua* in whole milk were pressurized in duplicate at 300, 350 and 400 MPa at ambient temperature (about 22 °C) for a total holding duration of 5, 10, 15 and 20 min for both continuous and pulsed HHP treatments. Experimental parameters are listed in Table III.1. The pressure holding time did not include the process come-up or depressurization times. After HHP treatment, enumeration of the micro-organisms was done as described above.

Table III.1. Experimental parameters for pulsed pressure treatment in whole milk

Pressure (MPa)	Temperature (°C)	Total holding time (min)	Pulse numbers for each total holding time
300, 350 and 400	20-25	5	5 min × 1 pulse 2.5 min × 2 pulses 1 min × 5 pulses 0.5 min × 10 pulses
300, 350 and 400	20-25	10	10 min × 1 pulse 5 min × 2 pulses 2 min × 5 pulses 1 min × 10 pulses
300, 350 and 400	20-25	15	15 min × 1 pulse 5 min × 3 pulses 3 min × 5 pulses 1.5 min × 10 pulses
300, 350 and 400	20-25	20	20 min × 1 pulse 10 min × 2 pulses 5 min × 4 pulses 4 min × 5 pulses 2 min × 10 pulses

Compression heating during pressure was taken into consideration as mentioned above in the experiments so that temperature of the pressure transmitting fluid during HHP treatment was controlled near ambient (~ 22 °C) temperature (the temperature increase of ethylene glycol-pressure transmitting fluid- and whole milk were about 4.0 °C/100 MPa and 3.4 °C/100 MPa, respectively). Typical pressure and temperature profiles for both continuous (5 min × 1 pulse) (Fig. III.4a) and pulse (1 min × 5 pulses) (Fig. III.4b) HHP treatments at 300 MPa are shown in Fig. III.4.

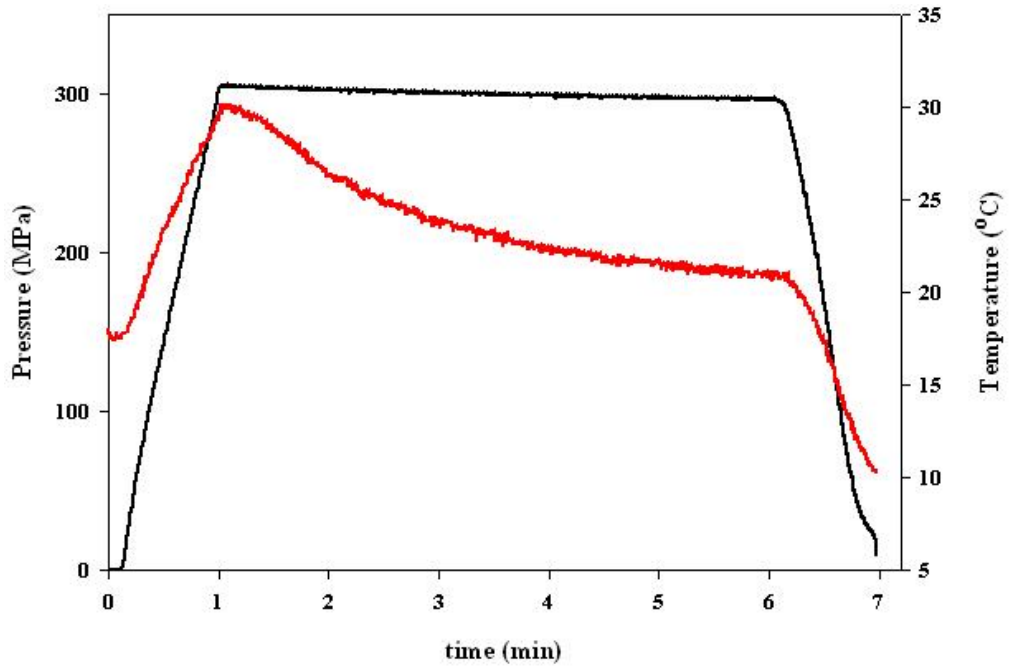


Fig. III.4a. Pressure (black lines) and temperature (red lines) profiles measured at 300 MPa for 5 min \times 1 pulse.

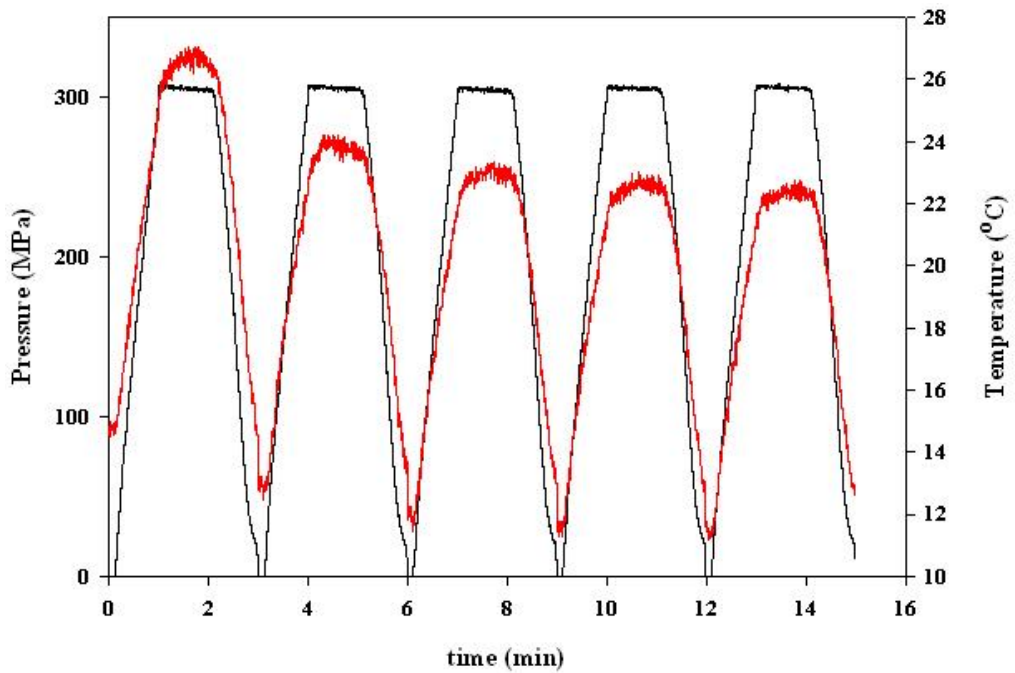


Fig. III.4b. Pressure (black lines) and temperature (red lines) profiles measured at 300 MPa for 1 min \times 5 pulses.

III.3.2.3) Microbial inactivation experiments in kiwifruit and pineapple juices

Kiwifruits and pineapples were obtained from a local market. Fruits were washed, peeled, cut and pulped using a hand-held bar kitchen blender. Then the pulp-juice mixtures of each fruit were filtered to remove the pulp. The juices obtained were further filtered to sterilize (Minisart[®], 0.45 μm , Sartorius, Goettingen, Germany) and stored at 4 °C until use. pH of the kiwifruit and pineapple juices were 3.32 and 3.77, respectively. Each juice was inoculated with pure cultures from the early stationary phase to obtain about 10^7 colony forming units (CFU) mL^{-1} juice at least 1 h before treatment to allow cells to adapt to the new environment. One mL of un-inoculated juices were transferred onto TSAYE plates (Sterilin, Staffordshire, UK) and incubated at 37 °C for 48 h to make sure that the juice samples were sterile.

In a first attempt, the strains were pressurized in duplicate at 300 MPa for 300 s at 20, 0 and -10 °C. Secondly, pulsed HHP treatment were applied at 300 MPa for a total duration of 300 s with different pulse number at 20 and 0 °C (150 s \times 2 pulses, 100 s \times 3 pulses, 75 s \times 4 pulses, 60 s \times 5 pulses, 50 s \times 6 pulses and 30 s \times 10 pulses; the last two pulse treatment were only done at 20 °C).

III.3.2.3.1) Storage of HHP treated juices

Samples of inoculated juices were pressure treated at 350 MPa for 60 s \times 5 pulses at 20 °C and held at 4, 20 and 37 °C for 28 days. At 1st, 7th, 14th and 21st days of storage 1 ml (0.3, 0.3 and 0.4 ml) of both juice were surface plated on pre-poured TSAYE. The plates were incubated at 37 °C for 72 h and examined for presence or absence of colony formation on agar plates.

III.4) Equipment for experiments with high pressure carbon dioxide (HPCD)

A carbon dioxide (CO₂) tube is connected to a HHP equipment using an indirect compression is shown in Fig. III.5 for experiments using carbon dioxide under high pressure. To reach high pressures (> 5 MPa) a compressor is connected between CO₂ tube and HHP equipment.



Fig. III.5. HHP equipment with indirect compression system used for HPCD experiments

III.4.1) HPCD experiments using carbon dioxide

In a first attempt HPCD experiments were performed in skim milk at 5, 250 and 300 MPa at room temperature by using the compressor and CO₂ tube. In a second attempt solid CO₂ was placed (40 mg of solid CO₂) in each 8 ml of plastic bottles) and pressurization was done at 400 MPa, 5 min × 1 pulse and 1 min × 5 pulses. In order to compare the efficiency of HPCD, the same experiments were also repeated without CO₂.

III.5) Statistical analysis of the data

Analysis of variance (ANOVA) as implemented in SPSS 10.0 for Windows (SPSS, Inc, Chicago, USA) was used to test effects of temperature, microorganisms and fruit juices on the logarithmic survival ratio. Tukey, Duncan and Student-Newman-Keuls post-hoc tests were used as paired comparisons between sample means. Level of significance was set to 0.05.

CHAPTER IV

EVALUATION OF THE COMPRESSION HEATING OF THE SELECTED PRESSURE TRANSMITTING FLUIDS AND LIQUIDS FOODS DURING HHP TREATMENTS

IV.1) Compression heating of selected pressure transmitting fluids and liquid foods during HHP treatment

IV.1.1) Effect of process variables on compression heating

Table IV.1 shows the temperature increase values per 100 MPa for all the samples. Among the substances studied, ethanol and ethylene glycol had the highest (up to 12.9°C/100 MPa) and the second highest (up to 4.9°C/100 MPa) compression heating values, respectively. Water was selected as a reference because its properties under pressure were well documented (Harvey et al. 1996).

Ethylene glycol is often used as a pressure transmitting fluid especially at low temperatures (Kalichevsky et al. 1995; Buzrul et al. 2007a), as it remains liquid at some subzero temperatures at atmospheric pressure. Ethanol is also used as a pressure transmitting fluid in mixtures with water (Rasanayagam et al. 2003).

Orange juice (up to 3.8°C/100 MPa), whole milk (up to 3.8°C/100 MPa), and skim milk (up to 3.7°C/100 MPa) presented similar compression heating values as that of water (up to 3.4°C/100 MPa). Liquid foods were chosen because of their differences in origin, composition, and pH. Orange juice was chosen as a model for food of plant origin. Its main characteristics were high concentrations of sugars, organic acids, and low pH (3.6). As a model of foods of animal origin skim and whole milk were selected. Both skim and whole milk had pH values around 6.7. The differences in fat contents between whole milk and other liquid foods did not affect the compression heating values. Given that all the liquid foods studied had high water content (~ 90%), their thermal behavior under pressure was closely comparable to that of water or each other. Although adiabatic heat of these foods was already reported in literature, the equipment used in this study is characterized by a larger pressure

vessel (3 L, diameter: ~ 10 cm and height: ~ 40 cm) than the ones used in others studies (Rasanayagam et al. 2003; Patazca et al. 2007).

Rasanayagam et al. (2003) has observed high compression heating values for ethanol (up to 10.6°C/100 MPa) and propylene glycol (up to 5.8°C/100 MPa). Ethylene glycol can be considered as a model for food, in terms of hydrophobicity, which lies between that of pure water and pure fat (Kalichevsky et al. 1995). Shimizu (1992), has reported that fat is characterized by a large temperature increment during HHP treatment. Similarly, Rasanayagam et al. (2003) and Patazca et al. (2007) have demonstrated that fats and oils were characterized by very high compression heating values. This may explain the second highest compression heating value of ethylene glycol among the substances studied.

The polarity of a substance can induce some electrostatic interactions between molecules and can lead to electrostatic bonding. Under HHP, as a result of the low energy applied and volume change ($\Delta V < 0$), such chemical bonds may be broken, leading to a new spatial arrangement between the molecules and energy could be released. However, it should be necessary to consider isothermal compressibility values. Ethanol has the highest compression heating most probably on account of its high value of isothermal compressibility.

Results indicated that, for all the samples studied, as the initial temperature of the substances was improved the compression heating values were also increased. This phenomenon was also observed by Patazca et al. (2007) for water, honey, and cream cheese. In general, as the pressure level increased (from 100 to 400 MPa) the improvement of the temperature per 100 MPa decreased for ethanol and ethylene glycol. However, the pressure level had no apparent impact on the temperature increase per 100 MPa in the case of the others substances studied (Table IV.1). Similarly, compression rate had an impact only on

Table IV.1. Temperature increase per 100 MPa [$100 \Delta T / \Delta P$ ($^{\circ}\text{C}/\text{MPa}$)] of selected pressure transmitting fluids and liquid foods at different pressure levels, initial temperatures and compression rates.

		$100 \Delta T / \Delta P$ ($^{\circ}\text{C}/\text{MPa}$)									
		$T_i = 5^{\circ}\text{C}$			$T_i = 20^{\circ}\text{C}$			$T_i = 35^{\circ}\text{C}$			
		Come-up rate (MPa/min)	100	200	300	100	200	300	100	200	300
		P (MPa)									
Water	100	1.4	2.2	1.7	2.6	2.5	2.2	3.1	3.0	3.0	
	200	2.3	2.6	1.7	2.7	2.6	2.6	3.1	3.2	3.3	
	300	2.3	2.2	2.2	2.8	2.2	2.7	3.2	3.2	3.3	
	400	1.8	1.9	2.4	2.6	2.6	2.8	3.2	3.1	3.4	
Ethanol	100	11.5	12.2	—	12.6	12.7	—	12.9	12.9	—	
	200	9.0	10.3	10.5	9.7	10.6	11.3	10.7	11.5	11.4	
	300	7.1	8.7	9.3	7.7	9.1	10.2	7.3	9.2	9.8	
	400	5.7	6.7	7.8	5.9	7.6	8.2	5.5	7.2	8.0	
Ethylene Glycol	100	3.6	4.5	4.3	4.9	4.3	4.1	3.7	4.2	4.5	
	200	3.8	3.9	4.0	4.2	4.1	4.2	3.3	4.0	4.4	
	300	3.3	3.7	3.6	3.7	3.9	4.1	2.6	4.0	—	
	400	2.8	3.3	3.5	3.7	3.6	3.7	3.2	3.4	—	
Whole Milk	100	1.9	1.3	1.4	2.7	2.5	2.1	3.7	3.3	3.5	
	200	2.3	2.5	2.5	3.1	3.2	3.1	3.8	3.5	3.5	
	300	2.3	2.5	2.5	3.1	3.3	3.2	3.6	3.9	3.7	
	400	2.1	2.6	2.6	2.9	3.0	3.3	3.7	3.9	3.6	
Skim Milk	100	2.0	1.8	1.6	2.6	2.7	2.4	3.2	3.5	3.7	
	200	2.4	1.6	1.7	2.6	2.6	2.7	3.6	3.6	3.3	
	300	2.3	2.2	2.2	2.8	2.9	2.5	3.4	3.6	3.6	
	400	2.0	2.4	2.4	3.0	3.1	3.1	3.4	2.7	3.0	
Orange Juice	100	1.8	1.4	1.5	2.7	2.8	2.6	3.8	3.2	3.2	
	200	2.1	1.9	2.2	3.0	3.0	3.0	3.1	3.6	3.5	
	300	2.3	1.9	2.9	2.7	2.8	2.9	3.5	3.4	3.6	
	400	2.3	2.8	2.4	2.7	2.7	2.8	3.2	3.1	3.3	

Data points are the average of three replicates and maximum standard error for the overall data is 0.5.

the compression heating values of ethanol and ethylene glycol. It may be expected that, as the compression rate increased the compression heating value was also improved (Makita et al. 1991; Kalichevsky et al., 1995), however, the results here indicated that water and liquid foods containing high amount of water were not affected by the compression rate within the range studied (100 to 300 MPa/min).

Typical pressure and temperature profiles for water, ethanol, and ethylene glycol are given in Fig. IV.1. Temperature of each substance was increased during compression. The temperature increased of about 20, 8, and 5°C for ethanol, ethylene glycol, and water, respectively. Given that density, heat capacity, and thermal expansivity of these substances are different, the change in temperature as a result of compression is different. Upon expansion, the temperature drops back to its initial value (20°C). Any variation in heat transfer characteristics of the pressure vessel (such as area/volume ratio of the pressure chamber, location of the temperature sensor within the vessel, and insulation properties of the pressure chamber material) is liable to yield a different temperature profile (Balasubramanian and Balasubramaniam 2003).

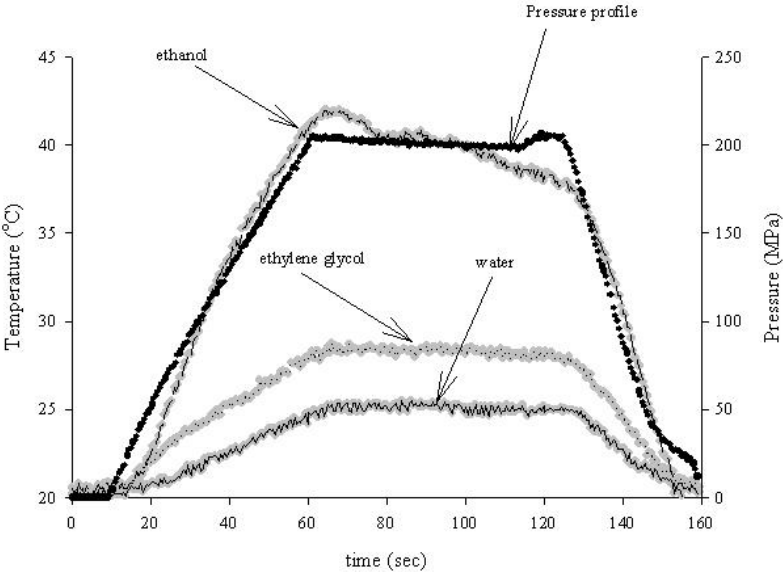


Fig. IV.1. Pressure and temperature profiles of water, ethylene glycol and ethanol. Initial temperature of the substances was 20 °C and compression rate was set to 200 MPa/min.

IV.1.2) Modeling the temperature increase as a function of initial temperature and pressure level

A second-order polynomial function [Eq.(4)] was proposed to describe the temperature rise (T) as a function of initial temperature (T_0) and pressure (P)

$$T(T_0, P) = a + bT_0 + cT_0^2 + dP + eP^2 + fT_0P \quad (4.1)$$

where a , b , c , d , e , and f are the parameters of Eq. (4.1).

A backward regression procedure was applied to determine the parameters of this equation. The procedure begins with all candidate variables in the function and then systematically removes variables that are not significantly associated with the target, until a model with only significant parameters is obtained. For the model assessment study, the adjusted regression coefficient (R^2_{adj}) value has been used as a quantitative way to measure the performance of the proposed model.

The proposed equation [Eq. (4.1)] was fitted to each sample studied at different initial temperature and pressure values. Figs. IV.2, IV.3, and IV.4 represent these resulting fittings for different pressure transmitting media as water or ethanol and liquid food as whole milk.

High regression coefficient (R^2_{adj}) values indicated reasonable fittings using this model (Table IV.2). It should also be noted that the proposed model involved six parameters and only 12 fitted data points; therefore its fitting was obvious. Nevertheless, by using the backward regression procedure only the significant parameters were retained, that is the number of parameters was reduced (in some cases, a model with only two adjustable parameters was obtained) and the equation with the highest fitting capacity was chosen. The parameters of the model for each substance studied are also given in Table IV.2. Eq. (4.1) can be used to calculate the temperature increase under different combinations of T_0 and P values, different from those tested, but are within the range of the experimental values (i.e., interpolation region). For example, temperature increase of water at a P of 250 MPa and T_0 of

15°C can be calculated as 6.1°C corresponding to 2.5°C per 100 MPa. In principle, this equation can be used to predict the compression heating values of other substances under the combined effect of T_0 and P .

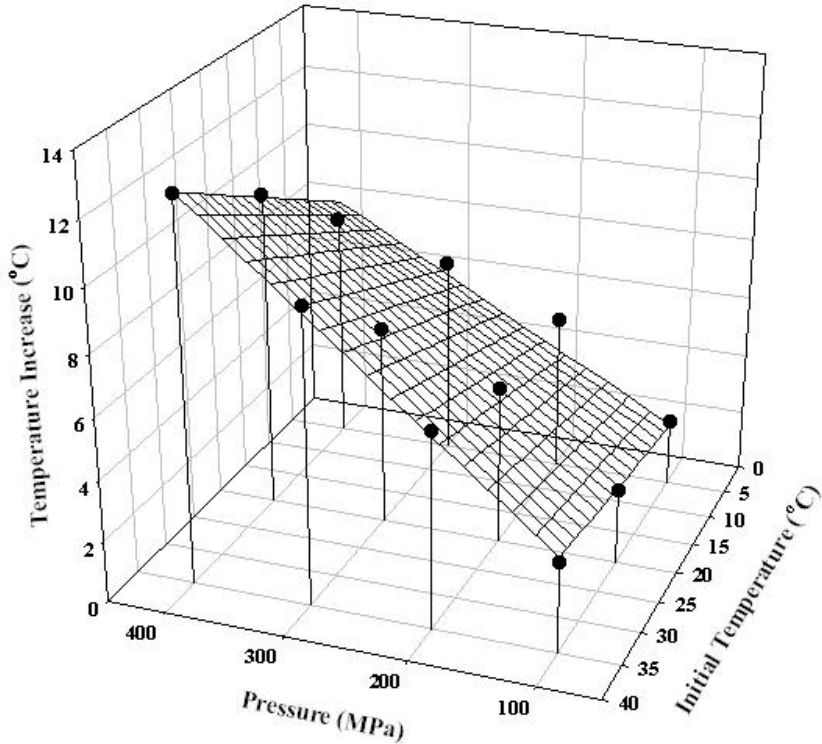


Fig. IV.2. Temperature increase of water fitted by Eq.(4.1). Black dots are the observed values.

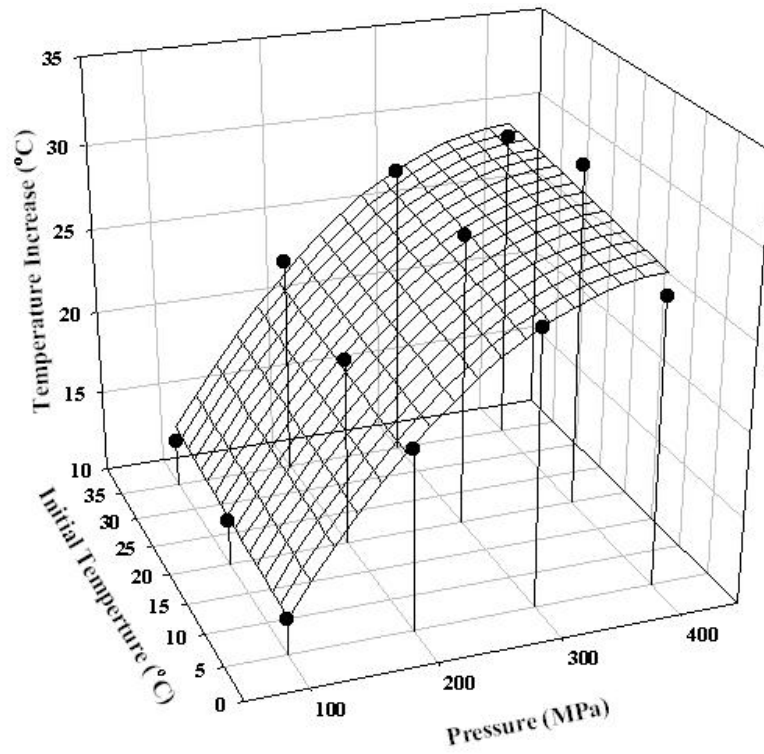


Fig. IV.3. Temperature increase of ethanol fitted by Eq.(4.1).
Black dots are the observed values.

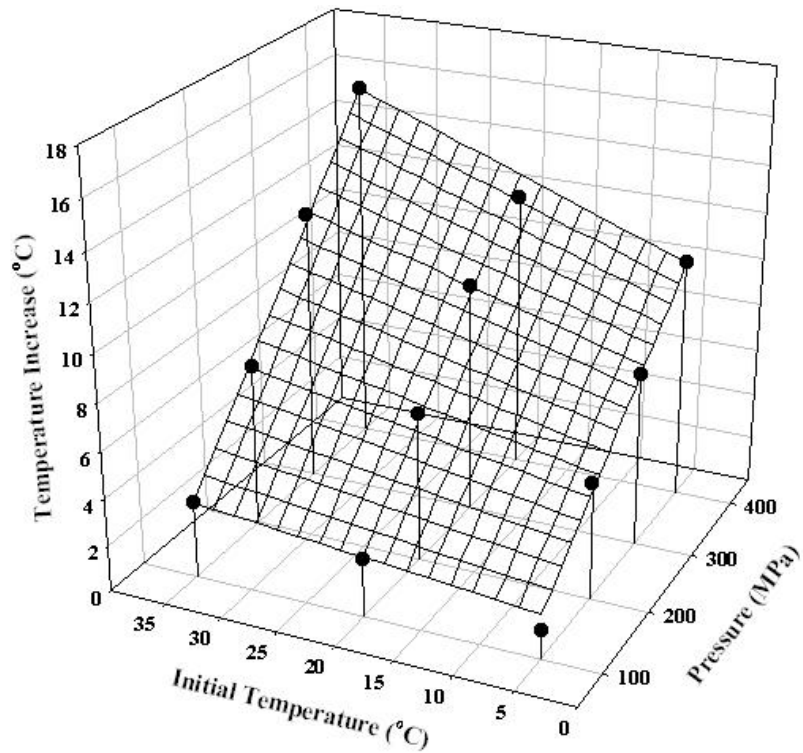


Fig. IV.4. Temperature increase of whole milk fitted by Eq. (4.1).
Black dots are the observed values.

Table IV.2. Parameters of Eq. (4.1) with their standard error values and adjusted regression coefficient (R^2_{adj}) values for each fit.

	Parameters	Eq. (4.1)	R^2_{adj}
Water	a	—	0.968
	b	—	
	c	—	
	d	0.02 ± 0.001	
	e	—	
	f	0.0003 ± 0.00001	
Ethanol	a	—	0.984
	b	0.05 ± 0.02	
	c	—	
	d	0.14 ± 0.005	
	e	-0.0002 ± 0.00001	
	f	—	
Ethylene glycol	a	—	0.973
	b	0.14 ± 0.05	
	c	-0.003 ± 0.001	
	d	0.033 ± 0.001	
	e	—	
	f	—	
Whole milk	a	-0.9 ± 0.3	0.989
	b	—	
	c	—	
	d	0.026 ± 0.001	
	e	—	
	f	0.0004 ± 0.00001	
Skim milk	a	-2.1 ± 0.8	0.935
	b	0.09 ± 0.02	
	c	—	
	d	0.03 ± 0.002	
	e	—	
	f	—	
Orange juice	a	-2.3 ± 0.6	0.960
	b	0.09 ± 0.02	
	c	—	
	d	0.03 ± 0.002	
	e	—	
	f	—	

This type of modeling was also proposed by Patazca et al. (2007) for vegetable oil, honey, and cream cheese at different initial temperature (1 to 70°C) and pressure values (150 to 600 MPa). Second-order polynomial function (six parameters) described the temperature rise of the vegetable oil and honey, even as the third-order polynomial function (nine parameters) was used for cream cheese. However, as the backward regression procedure was not used in this study, the proposed models contain more parameters than the one proposed in this study.

IV.2) Concluding remarks

Among the substances studied, ethanol had the highest compression heating values, possibly because of its high value of isothermal compressibility. All the liquid foods studied were characterized by similar compression heating values as that of water. It was found that, as the initial temperature of the substances increased, the compression heating values also increased. In general, as the pressure level is improved the elevation of temperature per 100 MPa decreased for ethanol and ethylene glycol; however, the pressure level had no apparent impact on the temperature increase per 100 MPa of the others substances studied. Similarly compression rate had an impact on the compression heating of ethanol and ethylene glycol only.

A second-order polynomial function was proposed to describe the temperature rise as a function of initial temperature and pressure. The fittings underlined that the proposed model could be used successfully to evaluate the temperature increase during HHP processing.

CHAPTER V

HIGH HYDROSTATIC PRESSURE INACTIVATION OF *ESCHERICHIA COLI* AND *LISTERIA INNOCUA* IN DIFFERENT LIQUID FOODS

V.1) HHP inactivation of *Escherichia coli* and *Listeria innocua* in whole milk

V.1.1) Experimental results of the HHP inactivation of *Escherichia coli* and *Listeria innocua* in whole milk

Table V.1 shows the inactivation values of both microorganisms in whole milk at 500 and 600 MPa for a 5 min treatment. These values indicated that HHP treatments (500 and 600 MPa for 5 min) did not totally inactivate both bacteria in whole milk.

Table V.1. HHP inactivation of *E. coli* and *L. innocua* in whole milk

	<i>E. coli</i>	<i>L. innocua</i>
500 MPa, 5 min	- 1.23 ^a	- 1.51
600 MPa, 5 min	- 3.57	- 3.60

^a values are given as log₁₀S(t).

In literature, several authors report that bacteria are more resilient in a complex matrix such as milk. Chen and Hoover (2003) for instance demonstrated a strong baroprotective effect on *Yersinia enterocolitica* in whole UHT milk, with inactivation levels 3.5-4.5 logs lower than in phosphate buffer when treated at 350-450 MPa/22 °C for 10 min.

V.1.2) Modeling of the high pressure inactivation of *Escherichia coli* and *Listeria innocua* in whole milk

Over the years, several models have been proposed to describe the non-linear survival curves such as the ones described by Cerf (1977), Bhaduri et al. (1991), Cole et al. (1993) and Peleg and Cole (1998). Among the non-linear models, perhaps, the most simple and flexible one is the Weibull model and recently it is gaining popularity.

Different forms of the Weibull model were presented in literature (van Boekel 2002; Buzrul and Alpas 2004); however, decimal logarithm form [Eq. (5.1)] seems suitable since its parameters have physical significance (Couvert et al. 2005):

$$\log_{10} S(t) = -\left(\frac{t}{\delta(P)}\right)^{n(P)} \quad (5.1)$$

where $S(t)$ is the survival ratio i.e., $S(t) = N(t)/N_0$, [$N(t)$ and N_0 are the number of survivors after an exposure time t and initial number of microorganisms (CFU mL⁻¹), respectively]. This model is characterized by two parameters; δ is high pressure (P) dependent and is called time of first decimal reduction (units in min or s) i.e., time needed to reduce the initial population, N_0 to $N_0/10$ and n is the pressure dependent shape parameter.

The main advantage of this model is its simplicity and robustness to describe both monotonic downward concave (shoulder) survival curves ($n > 1$) and monotonic upward concave (tailing) survival curves ($n < 1$). Traditional first-order model can be derived from Eq. (5.1) and corresponds to a special case ($n = 1$) of the Weibull model.

Although the Weibull model [Eq. (5.1)] is a simple and flexible model to describe microbial inactivation, it has two major drawbacks: (i) assessment of parameters requires a nonlinear regression; (ii) n value is structurally strongly correlated with δ values. Consequently, both parameters are dependent; an error on δ being balanced by an error on n in the same way. Such an autocorrelation causes certain instability of parameter estimates (Mafart et al. 2002). In addition, the Weibull model [Eq. (5.1)], with one more parameters, is intrinsically more complex than the traditional first order model (Chen and Hoover 2004). Thus, it seems worthwhile to fix n value characteristic to a strain for the overall data (whole set of kinetics for each microorganism), so that δ values can be estimated from a linear regression (Mafart et al. 2002). Therefore, Eq. (5.2) was proposed for non-linear survival curves of microorganisms ((Mafart et al. 2002; Chen and Hoover 2004; Corradini et al. 2005).

$$\log_{10} S(t) = -\left(\frac{t}{\delta(P)}\right)^{n_{fixed}} \quad (5.2)$$

where n_{fixed} is a constant.

For the model assessment study, regression coefficient (R^2) and mean square error (MSE) values were used as a quantitative tool to measure the performance of the models.

In a first attempt, Eq. (5.1) was used to describe the survival curves of *E. coli* and *L. innocua* at 400, 450, 500, 550 and 600 MPa. Fig. V.1 shows the n values of *E. coli* at these pressure values and solid line indicates the fixed n value (0.78) for the overall data. Fixed n value was the mean of n values at five pressure values [since the number of data in each pressure value is equal (except 400 MPa; it has one more data point than other pressure values), the average value was calculated]. In a second attempt, the survival curves of the bacteria were fitted with Eq. (5.2), i.e., Weibull model with fixed shape parameter.

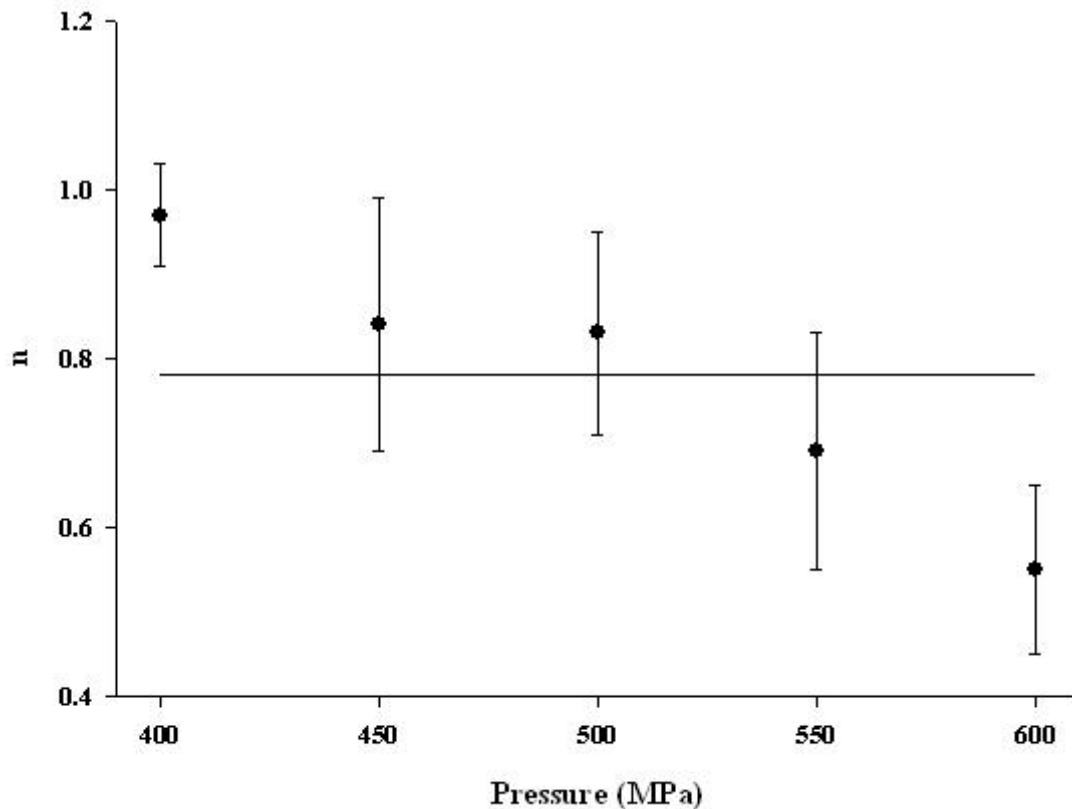


Fig. V.1. Shape parameters for the survival curves of *E. coli* ($n \pm 95\%$ confidence intervals of the Weibull model [Eq. (5.1)]. Solid line indicates fixed n value ($n = 0.78$) of the reduced Weibull model [Eq. (5.2)].

A disadvantage of using Eq. (5.1) is that every datum point is divided by the initial number of microorganisms [$S(t) = N(t)/N_0$]. This is equivalent with fixing the inoculum at the observed log count value. The loss is exactly the error in the initial number measurement. It would therefore in principle be better to use $N(t)$ rather than $S(t)$ and to estimate N_0 as a parameter. However, it turned out that when using three-parameter equation (N_0 , δ and n), δ behaved far from linear, causing very large and asymmetric confidence intervals (van Boekel 2002). Therefore, it was decided to use two-parameter equation [Eq. (5.1)] instead of three-parameter equation in this study.

Fig. V.2 shows the survival curves of *E. coli* (Fig. V.2a) and *L. innocua* (Fig. V.2b) in whole milk at five different pressure values. Note that, 20 min of pressurization was necessary to achieve more than 8 log reduction at 600 MPa for both microorganisms. This result was probably due to the protective impact of fats and proteins present in whole milk on microbial cells. Black et al. (2007) studied the baroprotective effect of milk constituents on *L. innocua* treated by HHP. According to these authors, phosphate and citrate may protect microorganisms against changes in pH during HHP treatment, whereas the divalent cations calcium and magnesium may protect cell membranes against HHP.

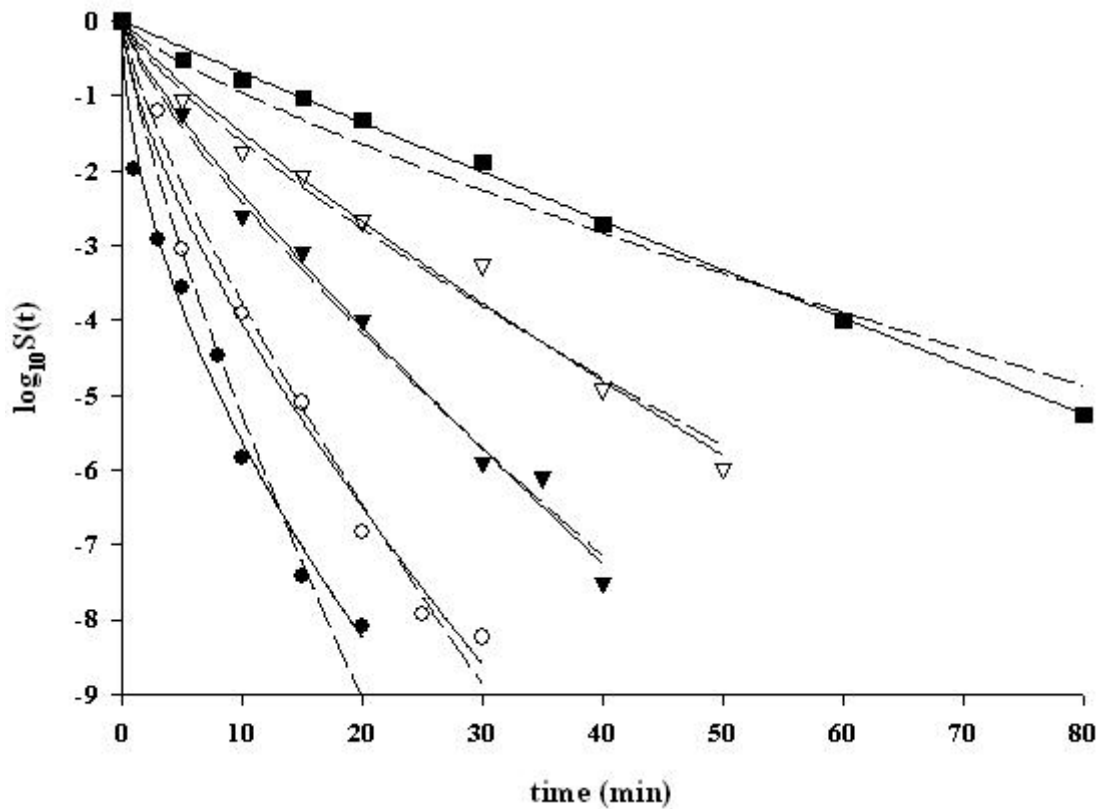


Fig. V.2a. Survival curves of *E. coli* ATCC 11775 in whole milk; (filled square): 400, (open reversed triangle): 450, (filled reverse triangle): 500, (open circle): 550 and (filled circle): 600 MPa. Solid lines indicate data were fitted with the Weibull model [Eq.(5.1)] with a variable n , dashed lines indicate data were fitted with the reduced Weibull model [Eq. (5.2)] with a fixed n ($n_{fixed} = 0.78$ for *E. coli*).

Solid lines in Fig. V.2 indicate the full model [Eq. (5.1)] and the dashed lines indicate the reduced model [Eq. (5.2)]. Visual inspection suggests that reduced form of the Weibull model can also be used to describe the inactivation of these microorganisms and support for this statement comes from the corresponding R^2 and MSE values (Table V.2).

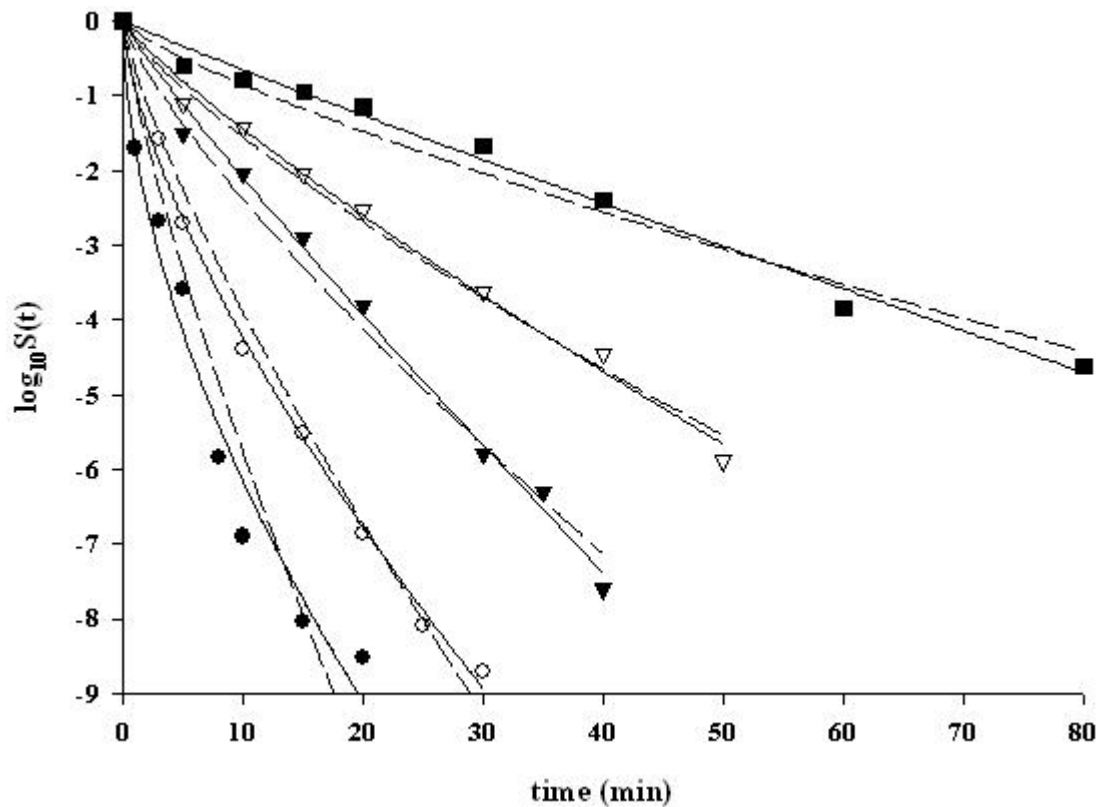


Fig. V.2b. Survival curves of *L. innocua* ATCC 33090 in whole milk; (filled square): 400, (open reversed triangle): 450, (filled reverse triangle): 500, (open circle): 550 and (filled circle): 600 MPa. Solid lines indicate data were fitted with the Weibull model [Eq.(5.1)] with a variable n , dashed lines indicate data were fitted with the reduced Weibull model [Eq. (5.2)] with a fixed n ($n_{fixed} = 0.79$ for *L. innocua*).

Table V.2. Goodness-of-fit of the Weibull [Eq. (5.1)] and the reduced Weibull [Eq. (5.2)] models

Microorganism	P (MPa)	R^{2a}		MSE^b	
		Eq. (5.1)	Eq. (5.2)	Eq. (5.1)	Eq. (5.2)
<i>E. coli</i>	400	0.99	0.98	0.008	0.07
	450	0.98	0.98	0.07	0.07
	500	0.99	0.99	0.06	0.06
	550	0.98	0.98	0.17	0.20
	600	0.99	0.93	0.12	0.50
<i>L. innocua</i>	400	0.99	0.98	0.03	0.06
	450	0.99	0.99	0.03	0.04
	500	0.99	0.99	0.05	0.09
	550	0.99	0.99	0.04	0.12
	600	0.98	0.92	0.28	0.75

^aRegression coefficient

^bMean Square Error

For the reduced model R^2 values were between 0.93 to 0.99 and 0.92 to 0.99 and MSE values were between 0.06 to 0.50 and 0.04 to 0.75 for *E. coli* and *L. innocua*, respectively. Although, there was a slight loss of goodness-of-fit by fixing the shape parameters of the survival curves of the microorganisms (n_{fixed} is 0.78 and 0.79 for *E. coli* and *L. innocua*, respectively) fixing of n causes a better stability of δ estimates and leads to an improvement of the robustness of the model (Couvert et al. 2005); in addition, process calculations are definitely easier and more reliable if n would not depend on pressure. This last point enables the modeling of the pressure dependence of the parameter δ by taking into account all the pressures studied at once in a single-step procedure, so that the number of degrees of freedom increases substantially for the estimation of δ value (van Boekel 2002). δ values obtained from the reduced Weibull model and their corresponding confidence intervals were also given in Table V.3.

Table V.3. Time constant parameter (δ) \pm 95 % confidence intervals and fixed shape parameter (n_{fixed}) of the reduced Weibull model [Eq. (5.3)] for *E. coli* 11775 and *L. innocua* ATCC 33090, respectively

P (MPa)	<i>E. coli</i>		<i>L. innocua</i>	
	n_{fixed}	δ (min)	n_{fixed}	δ (min)
400	0.78	10.5 \pm 1.1	0.79	12.2 \pm 1.3
450		5.4 \pm 0.5		5.7 \pm 0.4
500		3.2 \pm 0.2		3.3 \pm 0.2
550		1.8 \pm 0.2		1.8 \pm 0.1
600		1.2 \pm 0.2		1.1 \pm 0.2

Fig. V.2 also indicates that the traditional first-order model could give reasonable fittings at lower pressure values; however, such a model was inadequate to describe the data corresponding to the survival micro-organisms at 550 and 600 MPa (see n values for *E. coli* in Fig. V.1) since there is an obvious tailing (monotonic upward concavity) for both microorganisms. Therefore, it was not possible to use traditional first-order model for the all

the data gathered but by fixing the shape parameter, a model with a single parameter (just like traditional first-order model) could be obtained. As mentioned above, fixed shape parameters were less than one ($n < 1$ i.e., tailing or upward concavity).

This result may indicate that remaining members of the population have the ability to adapt to applied pressure (van Boekel 2002; Buzrul et al. 2005b). Therefore, survival curves of these micro-organisms fitted with Eq. (5.2) with a fixed n value which is less than one, can be interpreted as an evidence that sensitive members of the populations are destroyed at a relatively fast rate leaving behind the survivors of higher and higher resistance (van Boekel 2002; Avsaroglu et al. 2006). Noma et al. (2006) also observed tailing in the survival curves of *Escherichia coli* O157:H7 inactivated by HHP. When such survival *E. coli* O157:H7 cells in a tail portion were repropagated (tail-culture) and subjected to a second pressure treatment, the cells of the tail-culture exhibited higher barotolerance compared to those of the original culture. The presence of a genetically pressure resistant subpopulation is suggested to be responsible for this tailing effect.

It should be noted that the Weibull model is applicable to only iso-conditions (isothermal, isobaric, isoconcentration, etc.) (Buzrul 2007a). However, the microbial reduction during pressurization and expansion periods was not considered in this study. In fact, as the experiments were not performed under perfectly isobaric conditions (due to compression and decompression) and what fitted were the logarithms of the survival ratios not the survival ratios themselves, the relative weight of the small ratios is skewed (which is fine when dealing with microbial survival) and distribution's shape is slightly distorted (Buzrul et al. 2005b). It should be also kept in mind that during heat treatment significant reduction can occur during the come-up (heating to the target temperature) period; however, in HHP processing, a significant reduction could be possible after several minutes of holding time (Peleg 2002) at least for some pressure and temperature ranges.

Primary models [such as Eq. (5.1)] describe the change in bacterial counts with time under particular environmental conditions. Secondary models describe one or more parameters of a primary model under different environmental conditions such as pH, water activity, temperature and pressure. It is possible to describe the time constant parameter (δ) of the Weibull model with various empirical functions with two adjustable parameters. Hence, Eq. (5.3) was proposed to describe δ , determined from the survival curves fitted with fixed n values, as a function of pressure (P), analogous to the modeling of the classical D value in a so-called Thermal Death Time (TDT) curve:

$$\log_{10} \delta = a - b.P \quad (5.3)$$

where a and b are the parameters of Eq. (5.3). If so required, one could define z_p value (analogous to the classical z value). Then from Eq. (5.4) it follows that:

$$z_p = -\frac{1}{b} \quad (5.4)$$

Fig. V.3 shows the fitting of the secondary model [Eq. (5.3)] which describes the parameter δ in terms of high pressure (P). Fig. V.3 indicates decrease of δ values as the pressure increases just like traditional D value (see also δ values in Table V.3). For *E. coli*;

$$\log_{10} \delta = (2.89 \pm 0.26^a) - (0.0047 \pm 0.0006^a)P; R^2 = 0.99 \text{ and } MSE = 0.0009 \quad (5.5)$$

and from Eq. (5.5);

$$z_p = \frac{1}{0.0047} = 212.8 \text{ MPa} \quad (5.6)$$

For *L. innocua*;

$$\log_{10} \delta = (3.14 \pm 0.32^a) - (0.0052 \pm 0.0006^a)P; R^2 = 0.99 \text{ and } MSE = 0.0013 \quad (5.7)$$

and from Eq. (5.7);

$$z_p = \frac{1}{0.0052} = 192.3 \text{ MPa} \quad (5.8)$$

where superscript a is the 95 % confidence intervals. Analogous to the traditional z value, z_p value was defined as the increase in pressure that results in one log unit decrease of δ values. This parameter provides information on the pressure sensitivity of δ values.

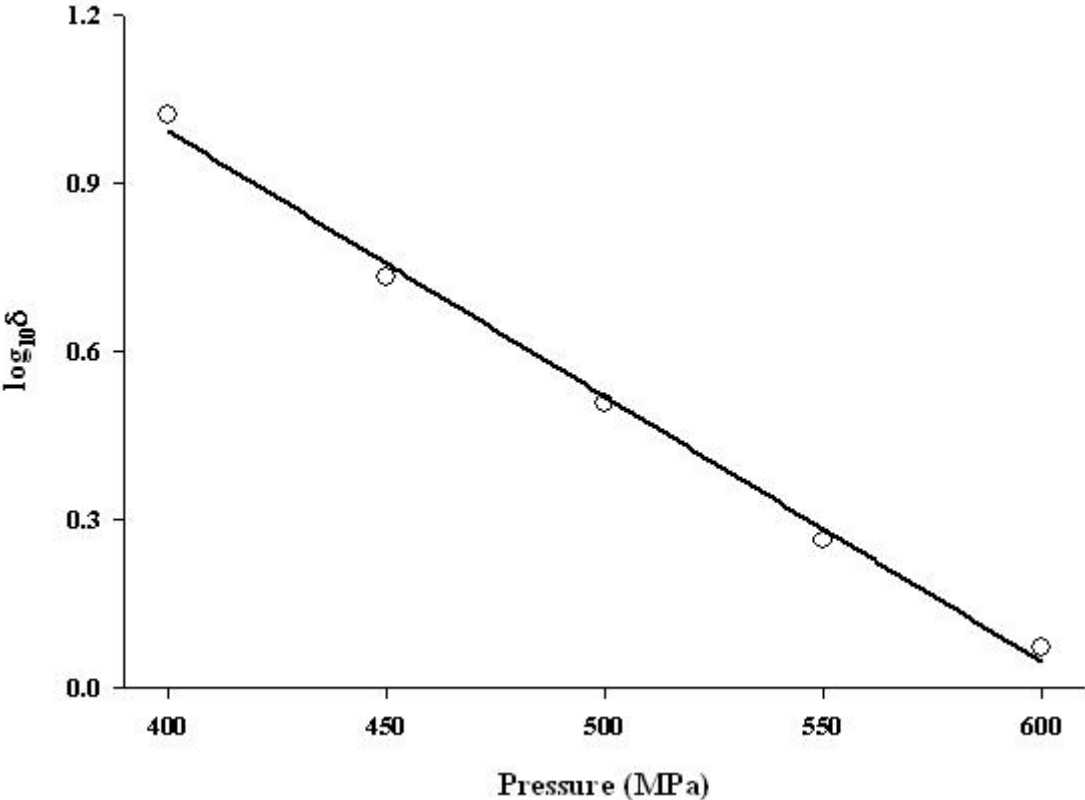


Fig. V.3a. Secondary modeling [Eq. (5.3)] of the parameter δ of the reduced Weibull model [Eq. (5.2)] for *E. coli* ATCC 11775.

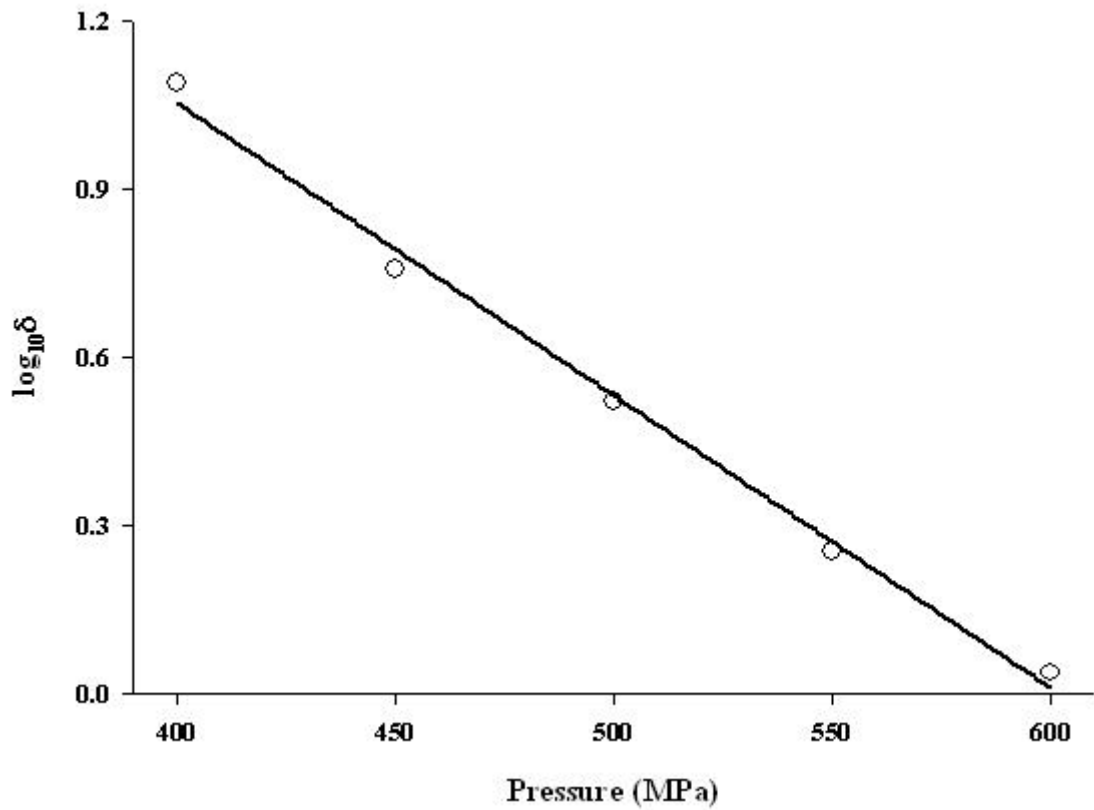


Fig. V.3b. Secondary modeling [Eq. (5.3)] of the parameter δ of the reduced Weibull model [Eq. (5.2)] for *L. innocua* ATCC 33090.

It should be noted that the proposed secondary model [Eq. (5.3)] is only applicable within the range 400-600 MPa therefore, extrapolation may cause inaccurate predictions. In literature, logarithms of δ values were described successfully with respect to temperature (Couvert et al. 2005; Fernández et al. 2007; Buzrul 2007b).

V.1.3) Efficiency of pulse pressure treatment for inactivation of *Escherichia coli* and *Listeria innocua* in whole milk

V.1.3.1) Pulse pressure treatments in the literature

Although milk is the first food product that has been pressurized (Hite 1899), up to date there is no commercially-pasteurized milk by HHP. This is due to baroprotective effect of milk on bacteria so that very high pressures are required for the inactivation of microorganisms. This effect was well documented in literature; milk fat content could provide a protective effect, but not a major one, since a considerable effect could be observed even in skim milk (García-Graells et al. 1999), the presence of calcium ions (Hauben et al. 1998) or solutes (Simpson and Gilmour 1997) may also confer protection. Recently, it was found that buffering by phosphate and citrate in milk may protect microorganisms against changes in pH during HHP treatment, whereas the divalent cations calcium and magnesium may protect cell membranes against HHP (Black et al. 2007).

Several studies have reported that greater microbial inactivation can be achieved by combining HHP with heat or antimicrobial compounds than by applying HHP alone (García-Graells et al. 1999; 2000; Alpas and Bozoglu 2000; Black et al. 2005). Such hurdle effects have many advantages in HHP processing including reduction of pressure levels to obtain desired target levels of microbial inactivation.

HHP treatment can consist of a single exposure period so-called continuous or static HHP (one pressure cycle; i.e., longer holding time) or the application of multi-pulsed pressure in shorter periods so-called pulsed or cycling pressure treatment (equal total holding duration but shorter holding times for each pulse). In literature, the efficiency of pulsed and continuous pressure treatments was compared. For example, López-Caballero et al. (2000) demonstrated that step-pulsed pressurization produced no apparent advantages over continuous

pressurization on the inactivation of microbial flora of the oysters. Similarly, Yuste et al. (2001) stated that pulse pressure treatment did not offer significantly better results than continuous pressurization of mechanically recovered poultry meat. On the other hand, Alemán et al. (1996) and Palou et al. (1998) observed higher microbial inactivation values for different combinations of pulsed pressure treatment than continuous one in pineapple juice and laboratory model system (Sabouraud glucose broth with sucrose), respectively. Pulsed pressure was also used in combination with antimicrobial peptides in milk (García-Graells et al. 1999). In the domain of pharmaceutical applications the comparison of continuous and pulsed HHP treatments has been investigated by Rigaldie (2002) for the inactivation of *Staphylococcus aureus* and *Bacillus subtilis*. Concerning food processing the role of pulsed pressure treatment has been underlined on the inactivation of two foodborne pathogens: *Staphylococcus aureus* and *Salmonella* Enteridis in a culture medium and inoculated into caviar samples (Fioretto et al 2005).

V.1.3.2) Impact of the different parameters characterizing pulse pressure treatments

V.1.3.2.1) Continuous pressure treatments

Fig. V.4 shows the effect of continuous HHP treatments at 300, 350 and 400 MPa for *E. coli* (Fig. V.4a) and *L. innocua* (Fig. V.4b) in whole milk. Results indicated that, at constant pressure increasing pressurization time from 5 to 20 min and at a constant holding time increasing pressure 300 to 400 did not increase cell death to a great extent. Inactivation of both microorganisms was only about one- \log_{10} at 400 MPa for 20 min. This result is expected since potential applications of HHP in milk (without additives and at room temperature) is more likely to be higher than 500 MPa (Chen and Hoover 2004; Buzrul et al. 2008).

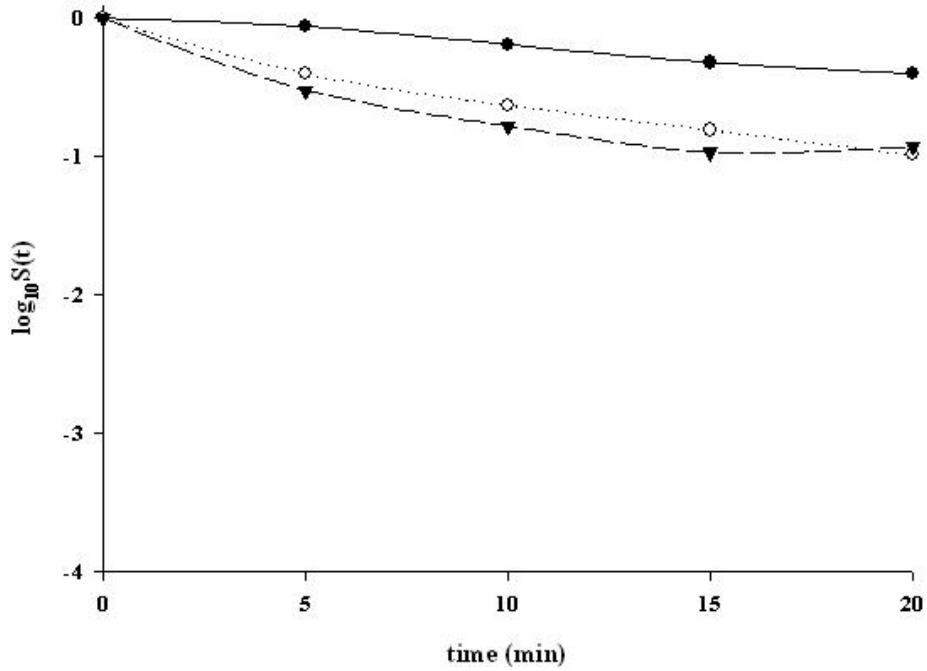


Fig. V.4a. Levels of inactivation of *E. coli* in whole milk by continuous HHP treatments; (filled circle): 300 MPa, (open circle): 350 MPa and (filled reversed triangle): 400 MPa.

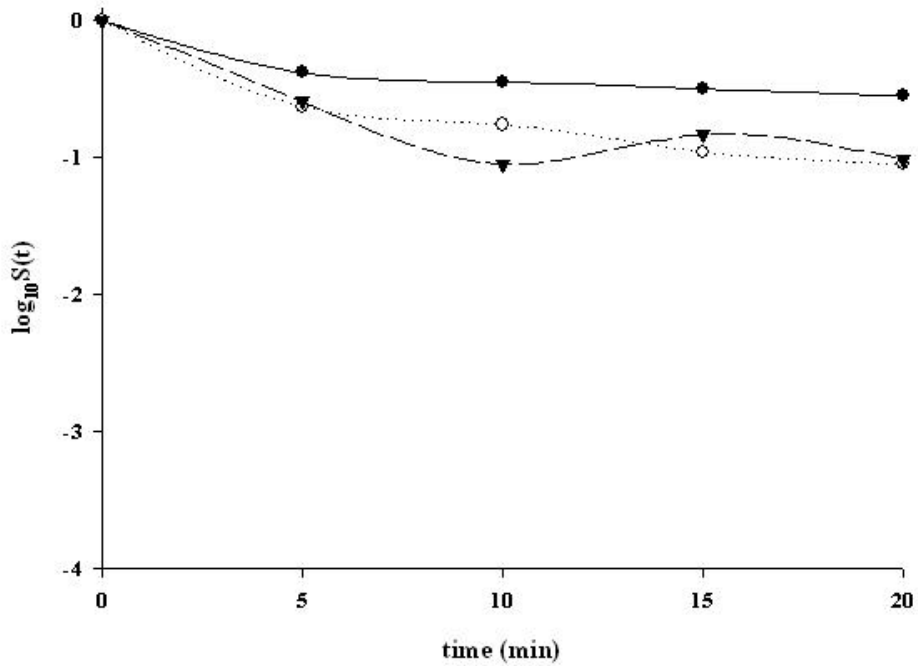


Fig. V.4b. Levels of inactivation of *L. innocua* in whole milk by continuous HHP treatments; (filled circle): 300 MPa, (open circle): 350 MPa and (filled reversed triangle): 400 MPa.

V.1.3.2.2) The pressure value

Fig. V.5 shows the effect of pulsed pressure treatments at 300, 350 and 400 MPa for a total holding duration of 10 min (1 min \times 10 pulses). Both bacteria had equal resistance to continuous and pulsed HHP treatments (Figs. V.4 and V.5). Fig V.5 indicates that even at the lowest pressure value, inactivation was higher than one- \log_{10} (close to two- \log_{10}) for both microorganisms showing the effectiveness of pulsed treatment over continuous treatment. Moreover, increasing pressure level from 300 to 400 MPa in pulsed pressure treatment significantly increases the microbial inactivation ($p < 0.05$); 4 \log_{10} reduction is possible at 400 MPa. This phenomenon was also observed by Palou et al. (1998) for the inactivation of *Zygosaccharomyces bailii* by pulsed HHP (increasing pressure from 207 to 276 MPa).

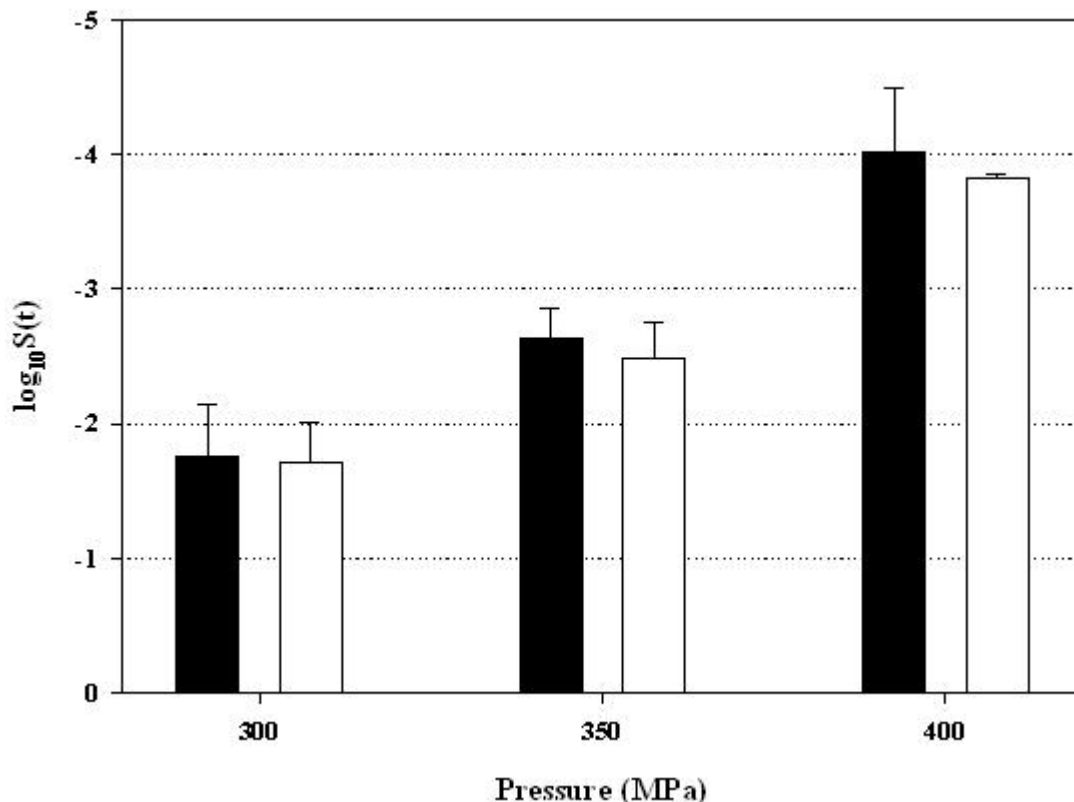


Fig. V.5. Levels of inactivation of *E. coli* (black color) and *L. innocua* (white color) in pulse HHP treatment (1 min \times 10 pulses) at different pressure levels. Error bars represent 95 % confidence intervals.

V.1.3.2.3) Effect of pulse number

Fig. V.6 shows the effect of pulsed treatments at 350 (Fig. V.6a) and 400 MPa (Fig. V.6b). The results indicated that as the pulse number increases (from 1 to 4 for pulse duration of 5 min) inactivation is increased. Again a comparison can be made with continuous HHP treatment; increasing the pressurization time from 5 min (5 min \times 1 pulse) to 20 min (5 min \times 4 pulses) increases cell death significantly ($p < 0.05$) in pulsed HHP treatment. It was also observed that the effect of pulse number is not additive. This may be due to the pressure resistance variation of each cell in a population; when all the weak members of the population are destroyed no further lethality occurs even if the number of pulses is increased. Moreover, repetition of compression and decompression phases may change the distribution of cell resistance and leave survivors of higher resistance behind (Donsi et al. 2007).

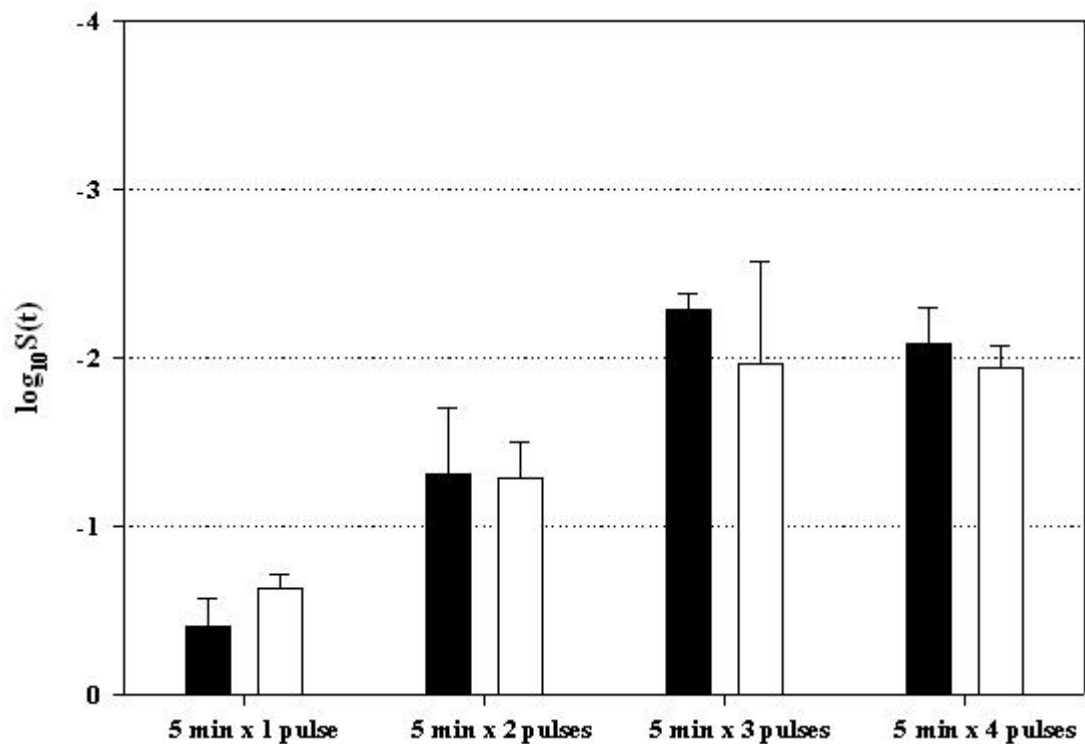


Fig. V.6a. Levels of inactivation of *E. coli* (black color) and *L. innocua* (white color) in different pulse HHP treatments (pulse holding time = 5 min) at 350 MPa. Error bars represent 95 % confidence intervals.

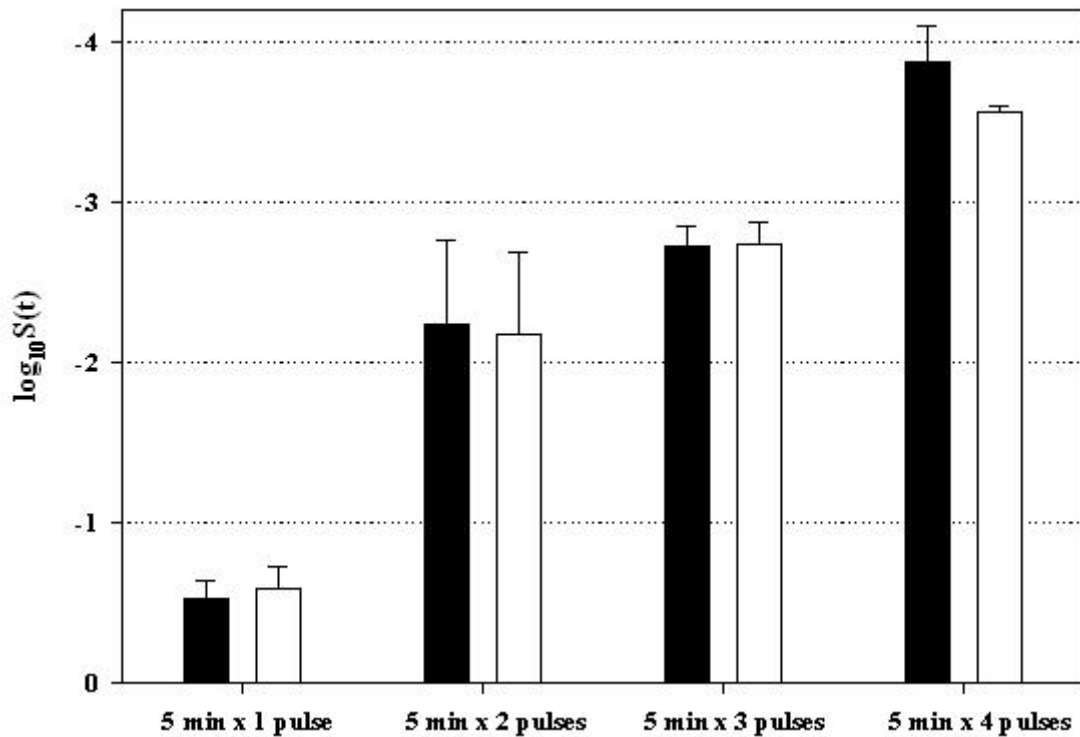


Fig. V.6b. Levels of inactivation of *E. coli* (black color) and *L. innocua* (white color) in different pulse HHP treatments (pulse holding time = 5 min) at 400 MPa. Error bars represent 95 % confidence intervals.

V.1.3.2.4) Effect of holding time

Choosing a total holding time of 20 min at 400 MPa, different combinations of pulse holding time and number of pulses were applied (20 min × 1 pulse, 10 min × 2 pulses, 5 min × 4 pulses, 4 min × 5 pulses and 2 min × 10 pulses). Fig. V.7 shows that the lethality of pulsed pressure treatment depends on the combination of pulse holding time and number of pulses tested at a given total holding time. As the number of pulses increases (up to 10 pulses) and the pulse holding time decreases inactivation increases. More than 4 log₁₀ reduction is possible at 400 MPa for 2 min × 10 pulses for both *E. coli* and *L. innocua*. Fig. V.7 also indicates that instead of one-pulse (continuous) HHP treatment, two pulses treatment with the same holding time could greatly enhance the microbial inactivation.

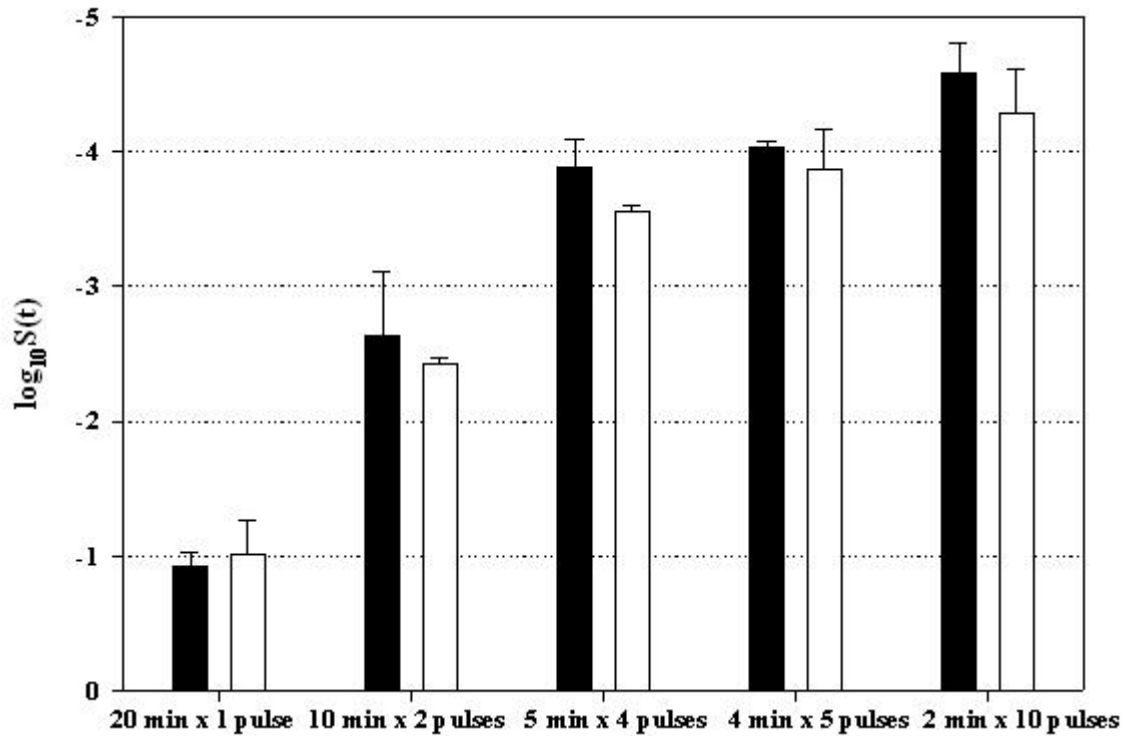


Fig. V.7. Levels of inactivation of *E. coli* (black color) and *L. innocua* (white color) in different pulse HHP treatments (total holding time duration = 20 min) at 400 MPa. Error bars represent 95 % confidence intervals.

In a second attempt, constant pulse number (5 pulses) was selected and duration of each pulse was increased from 1 to 4 min (Fig. V.8). Although increasing pulse duration from 2 to 3 min did not have a significant impact ($p < 0.05$), increasing the pulse duration could increase the microbial inactivation.

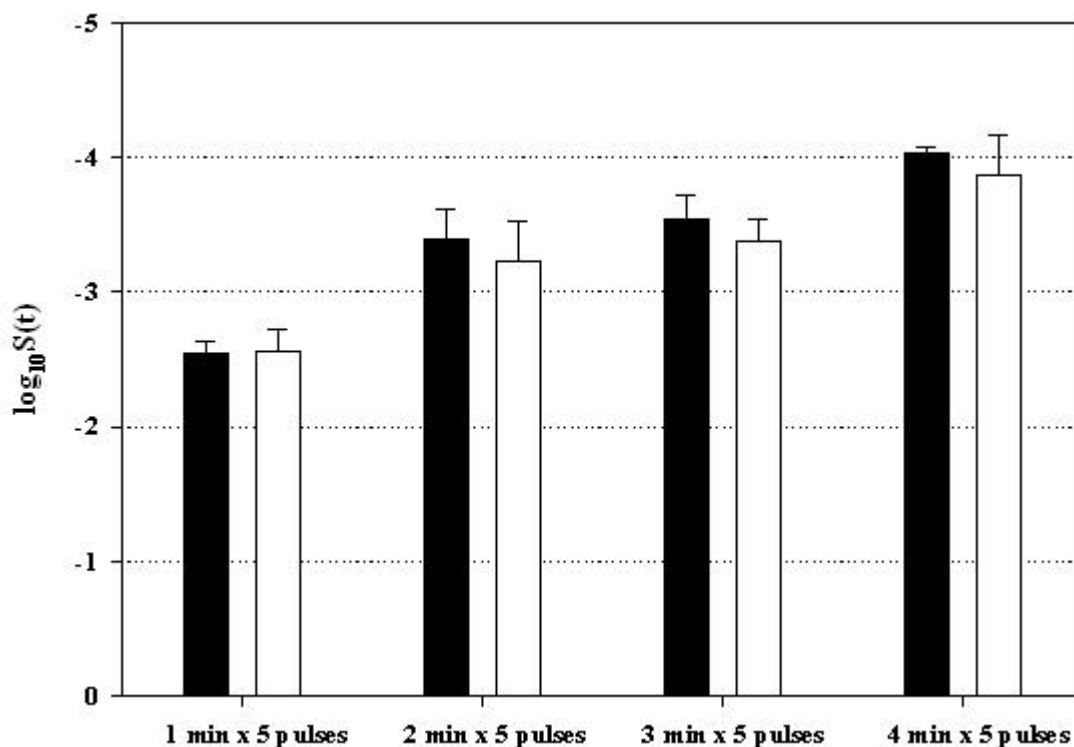


Fig. V.8. Levels of inactivation of *E. coli* (black color) and *L. innocua* (white color) in different pulse HHP treatments (pulse number = 5) at 400 MPa. Error bars represent 95 % confidence intervals.

V.2) HHP inactivation of *Escherichia coli* and *Listeria innocua* in fruit juices (kiwifruit and pineapple)

V.2.1) Challenge of HHP inactivation of microorganisms in fruit juices

Consumer demand for freshly-squeezed fruit juices is increasing, but such products are susceptible to spoilage and thus have a limited shelf-life (Jordan et al. 2001). HHP can give a response to the increasing consumer demand for fresh and minimally processed food products.

Kiwifruit can be considered as a highly nutritional product due to its high level of vitamin C content and its strong antioxidant capacity. Based on these characteristics, kiwifruit offers benefits for specific health conditions and has a great potential for industrial applications (Cassano et al. 2006); however, kiwifruit juice has no market worldwide. On the contrary, pineapple juice has been on the market shelves for some years, principally because

of its pleasant unique aroma and flavor. Nevertheless, the flavor of pineapple fruit is extremely sensitive to changes taking place during heat treatment (de Barros et al. 2003).

Some strains of *E. coli*, including the pathogenic O157:H7 strain, are acid-resistant and can survive for long periods in acid foods, especially at low temperature (Glass et al. 1992; Miller and Kaspar 1994; Jordan et al. 2001). *Listeria* spp. is not known to have caused outbreaks through the consumption of fruit juices but has been isolated from unpasteurized apple juice (Sado et al. 1998).

V.2.2) Impact of the different parameters characterizing the HHP treatments

V.2.2.1) Effect of the temperature

Fig. V.9 shows the 5 min HHP treatment (300 MPa) at three temperature values. Experimental data indicate that both bacteria had almost equal resistance to HHP. This was also reported by Buzrul et al. (2008) in whole milk for the same strains of these bacteria. It was also observed that microbial inactivation in kiwifruit juice was greater than the inactivation in pineapple juice treated by HHP. Moreover, temperature had no significant ($p > 0.05$) impact on the microbial inactivation; i.e., using low (0 °C) or sub-zero (– 10 °C) temperatures instead of room temperature (20 °C) during pressurization did not change the effectiveness of HHP treatment. In literature conflicting data exist about the high pressure low temperature processes. Yuste et al. (2002), for example, reported that pressurization at 20 °C is more lethal than at – 20 °C for the inactivation of mesophilic and psychrotrophic microflora of mechanically recovered poultry meat. On the other hand, Moussa et al. (2006) observed that pressurization, in the range of 100 to 300 MPa, at – 20 °C (in the liquid state) is more lethal than at 25 °C for inactivation of *E. coli*; however, at higher pressures this trend was reversed.

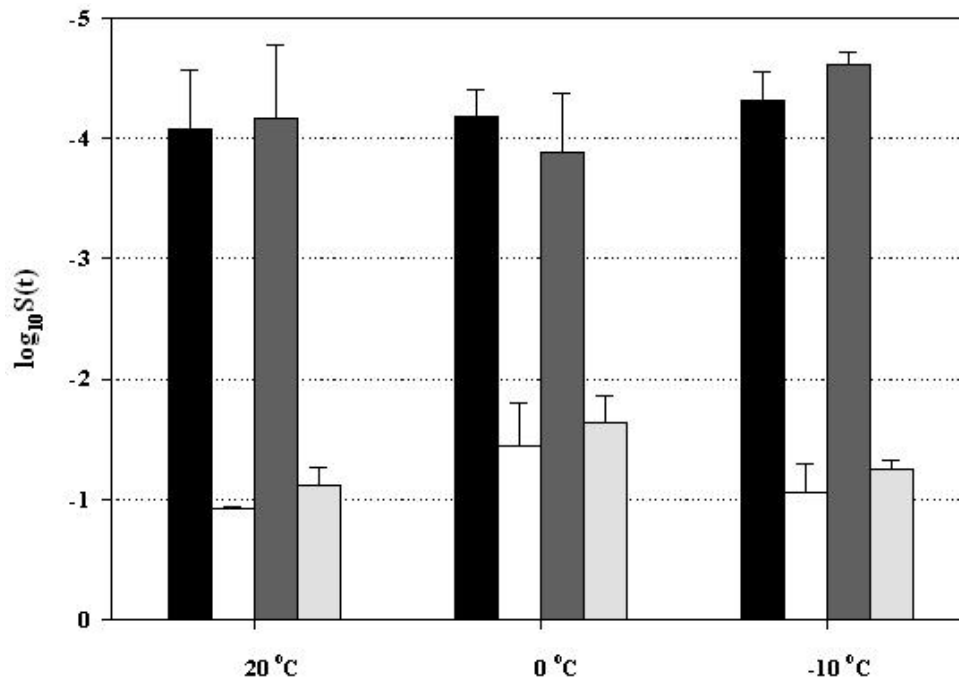


Fig. V.9. Inactivation levels of *E. coli* (black color in kiwifruit juice, white color in pineapple juice) and *L. innocua* (dark gray color in kiwifruit juice, light gray color in pineapple juice) in fruit juices at 300 MPa for 5 min at different temperature values. Error bars represent 95 % confidence intervals.

V.2.2.2) Effect of pulse pressure values

Different combinations of pulse holding times and number of pulses were applied with a total holding time of 5 min at 300 MPa (300 s × 1 pulse, 150 s × 2 pulses, 100 s × 3 pulses, 75 s × 4 pulses, 60 s × 5 pulses, 50 s × 6 pulses and 30 s × 10 pulses; the last two pulses treatment were only done at 20 °C) at 20 and 0 °C. Fig. V.10 shows HHP treatment at 20 °C up to 10 pulses at 300 MPa for 5 min; increasing the pulses number did not effect the microbial inactivation to a great extent in kiwifruit juice; however, in pineapple juice pulse treatment, especially after 5 pulses, increased the inactivation significantly ($p < 0.05$) for both bacteria. Fig. V.11 shows HHP treatment at 0 °C up to 5 pulses treatment. The same observation is also valid for this treatment.

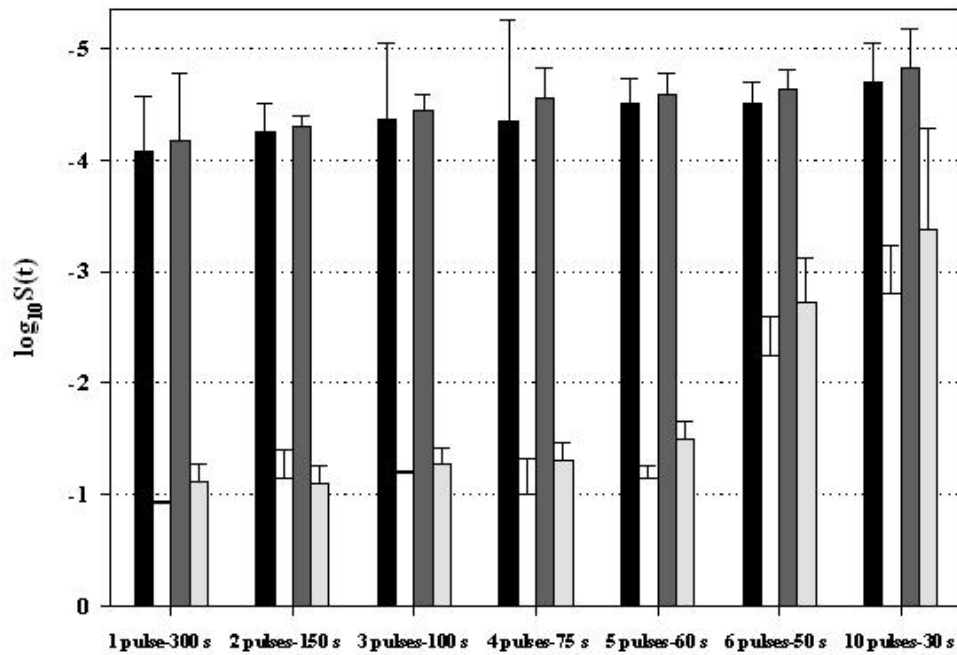


Fig. V.10. Inactivation levels of *E. coli* (black color in kiwifruit juice, white color in pineapple juice) and *L. innocua* (dark gray color in kiwifruit juice, light gray color in pineapple juice) in fruit juices at 300 MPa, 20 °C for different pulse numbers. Error bars represent 95 % confidence intervals.

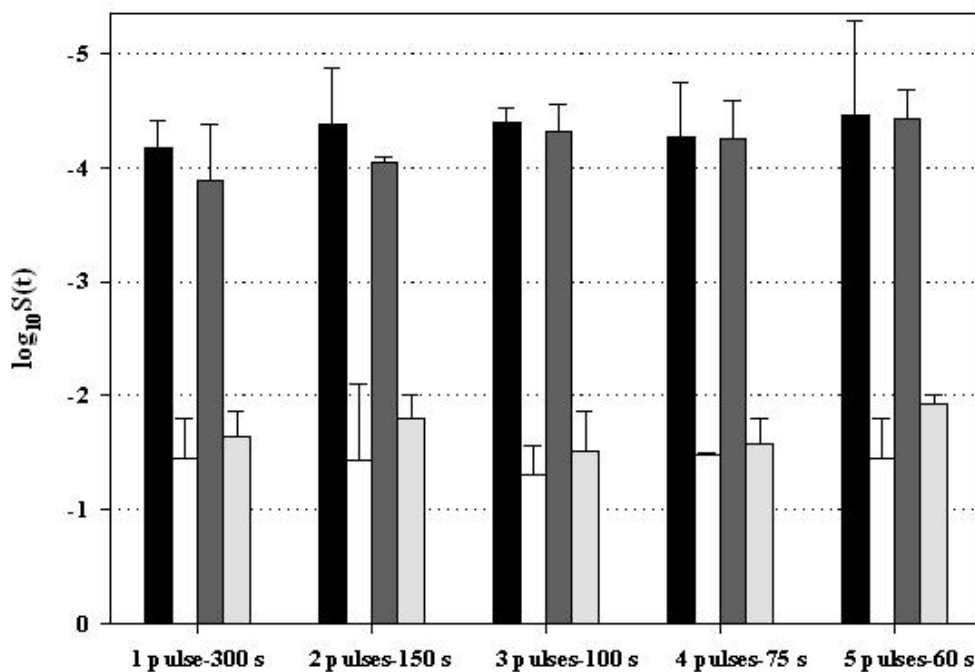


Fig. V.11. Inactivation levels of *E. coli* (black color in kiwifruit juice, white color in pineapple juice) and *L. innocua* (dark gray color in kiwifruit juice, light gray color in pineapple juice) in fruit juices at 300 MPa, 0 °C for different pulse numbers. Error bars represent 95 % confidence intervals.

The differences between kiwifruit and pineapple juices during pulse HHP treatment could be explained by the variation of resistance of each cell in a population. When all the weak members of the population are destroyed (leaving behind survivors of higher resistance) no further lethality occurs even if the number of pulses is increased (Donsì et al. 2007). Most probably, all the weak members were destroyed in kiwifruit juice even at single pulse HHP treatment hence increasing the pulse number (up to 10 pulses) did not effect the microbial inactivation, but increasing the pressure level may have some effect. In pineapple juice inactivation increased especially after 5 pulses treatment, indicating that some of the resistant cells of initial microbial population can be destroyed by increasing the number of pulses and by decreasing pulse holding time.

V.2.3) Role of storage and effect of storage temperature

In kiwifruit juice more than 5 log₁₀ reduction was obtained at 350 MPa, 20 °C for 60 s × 5 pulses immediately after HHP treatment; while about 2.5 and 3.5 log₁₀ reductions were observed in pineapple juice for *E. coli* and *L. innocua*, respectively. Inactivations were further increased more than 1 log₁₀ during storage at 4 °C for 24 h for both bacteria in both juices (Fig. V.12); for kiwifruit juice no viable cells were observed (7 log₁₀ reduction; complete inactivation) after 24 h at 4 °C. This phenomenon (inactivation increase during storage after HHP treatment) was also observed by Jordan et al. (2001) for *E. coli* in orange, tomato and apple juices.

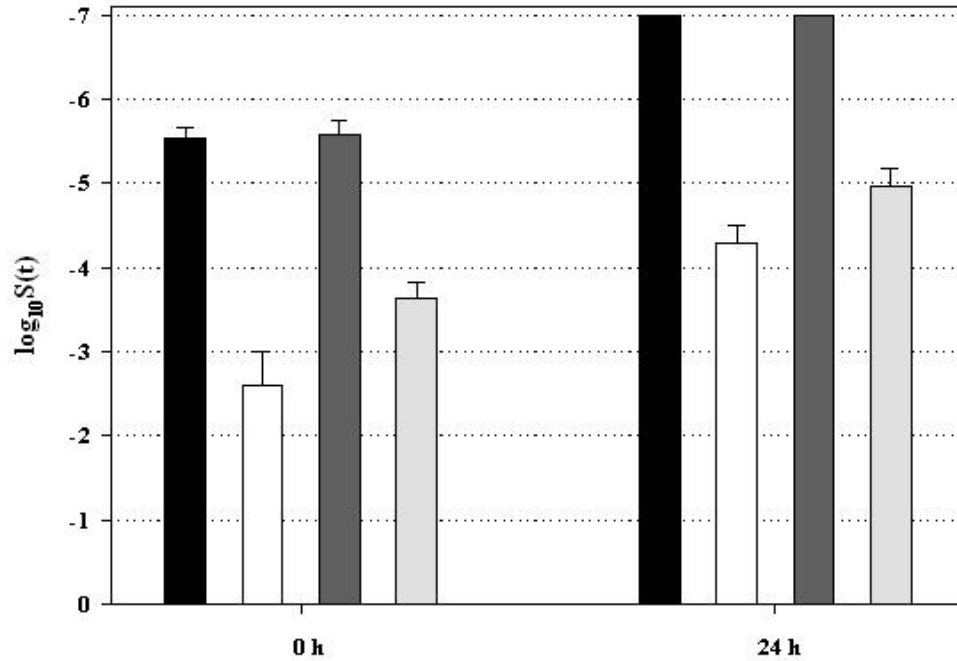


Fig. V.12. Inactivation levels of *E. coli* (black color in kiwifruit juice, white color in pineapple juice) and *L. innocua* (dark gray color in kiwifruit juice, light gray color in pineapple juice) in fruit juices at 350 MPa, 20 °C for 60 s × 5 pulses at 0 h and after 24 h at 4 °C. Error bars represent 95 % confidence intervals.

The effect of storage temperature on the recovery of *E. coli* and *L. innocua* after HHP treatment (350 MPa, 20 °C for 60 s × 5 pulses) was also tested. Table V.4 shows the effect of storage temperature during a period of 21 days. Viability of both bacteria in both juices was reduced by more than 7 \log_{10} at all storage temperatures (except 4 °C in pineapple juice) during the first 24 h. The subsequent storage enhanced further inactivation even at 4 °C and no recovery of the bacteria were detected during three weeks of storage at 4, 20 and 37 °C. This result indicates that the HHP treatment caused sublethal injury to a large proportion of the cells, resulting in a reduced resistance to low pH (Garcia-Graells et al. 1998) during storage; however, storage temperature is an important factor. In literature, it was reported that refrigeration enhances survival of *E. coli* in acidic environments (Zhao et al. 1993; Miller and Kaspar 1994; Conner and Kotrola 1995) which was confirmed in this study after HHP

treatment for both *E. coli* and *L. innocua* for the first 24 h at 4 °C when compared with 20 and 37 °C.

Table V.4. Recovery of *E. coli* and *L. innocua*^a during storage at different temperatures

	kiwifruit juice			pineapple juice		
	4 °C	20 °C	37 °C	4 °C	20 °C	37 °C
storage duration (day)						
1	- ^b	-	-	+	-	-
7	-	-	-	-	-	-
14	-	-	-	-	-	-
21	-	-	-	-	-	-

^a Table is valid for both bacteria

^b Presence (+) and absence (-) of colonies in 1 mL of fruit juices.

V.3) High pressure carbon dioxide (HPCD) inactivation of *E. coli* and *L. innocua* in skim milk

V.3.1) Role of CO₂ in high pressure treatments

For more than two decades now, the use of HPCD has been proposed as an alternative cold pasteurization technique for foods. This method presents some fundamental advantages related to the mild conditions employed, particularly because it allows processing at much lower temperature than the ones used in thermal pasteurization (Garcia-Gonzalez et al. 2007).

Almost every study found in literature concerning microbial inactivation by HPCD reports the influence of pressure and temperature on the inactivation efficiency. In general, microbial inactivation is accelerated with increasing CO₂ pressures. As a consequence, at higher pressures, a shorter exposure time is needed to inactivate the same level of microbial cells (Lin et al. 1993; 1994; Hong et al. 1997; Hong and Pyun 1999). Principally, pressure controls both the solubilization rate of CO₂ and its total solubility in a suspending medium. Therefore, a higher pressure enhances CO₂ solubilization to facilitate both acidification of the external medium as well as its contact with the cells. In addition, CO₂ at higher pressures in general exhibits a higher solvating power. The stimulating effect of CO₂ pressure, however, does not go on indefinitely and is limited by the saturation solubility of CO₂ in the suspending medium (Sims and Estigarribia 2003). Spilimbergo (2002) demonstrated that above 10 MPa the solubility of CO₂ was a weak function of pressure. An increase of pressure from 10 to 30 MPa at 55–60 °C did not influence appreciably the solubility of CO₂ in water; however, from an economical point of view, it increases significantly both operating and investment costs (Spilimbergo et al. 2005).

The microbial inactivation is also sensitive to the applied temperature. In general, the inactivation rate increases with increasing temperature (other conditions being equal). Higher

temperatures stimulate the diffusivity of CO₂ and can also increase the fluidity of the cell membrane to make penetration easier (Lin et al. 1993; Hong et al. 1997; Hong and Pyun 1999). However, HPCD treatment should not be operated at temperatures far above its critical temperature (certainly in the vicinity of the critical pressure of CO₂), because within this region the density of the solvent and hence its solubilization capacity decreases quite rapidly as temperature increases (Lin et al. 1993; Hong and Pyun 1999; Lucien and Foster 1999). So the stimulating effect temperature has on CO₂ penetration can in part be counteracted by its inhibiting effect on CO₂ solubility. HPCD treatments should also not be performed at too high temperatures because they could deteriorate the food quality in many applications (Lin et al. 1992; 1993; 1994; Hong et al. 1997; Hong and Pyun 1999).

V.3.2) Experimental results and discussion

Table V.5 shows the inactivation data of *E. coli* and *L. innocua* in skim milk by HPCD treatment at 5 MPa, 25 °C for 10 and 60 min. These data indicate that inactivations were less than one log₁₀ even for 60 min treatment at 5 MPa, 25 °C. This finding is consistent with other studies in literature. For example, Erkmén (2001) obtained about one and two log₁₀ reductions for *E. coli* at 10 MPa, 30 °C for 360 min in skim and whole milk, respectively.

Table V.5. Inactivation data of two bacteria in skim milk by HPCD at 5 MPa, 25 °C

	<i>E. coli</i>	<i>L. innocua</i>
10 min	-0.08 ^a	-0.03
60 min	-0.15	-0.32

^a values are given as log₁₀S(t).

To increase the efficacy of HPCD treatment further high pressure values (≥ 250 MPa) at 5 min were used (Table V.6) and inactivation data was compared with HHP treatment. As indicated by the data even at high pressure (250 and 300 MPa, 25 °C for 5 min) CO₂ did not

have great inactivation effects for both bacteria. Nevertheless, for the same pressure level HPCD treatment was more efficient than HHP treatment for both bacteria in skim milk.

Table V.6. Inactivation data of two bacteria in skim milk by HPCD at various pressure values, 25 °C for 5 min in comparison with HHP inactivation

	<i>E. coli</i>	<i>L. innocua</i>
HPCD 250 MPa	-0.61 ^a	-0.55
HPCD 300 MPa	-0.80	-0.60
HHP 300 MPa	-0.22	-0.28

^a values are given as log₁₀S(t).

For the last step, 40 mg of solid CO₂ was placed into 8 ml of plastic bottles filled with skim milk and then pressurized at 400 MPa, 25 °C for 5 min × 1 pulse and 1 min × 5 pulses and these values were compared with HHP treatment (Table V.7). It could be observed that at 400 MPa HPCD inactivations of both bacteria was higher than one log₁₀ which is significantly ($p < 0.05$) higher than HHP inactivation at the same pressure value. The efficacy of HPCD inactivation further increased when combined with pulsed treatment; about 4 log₁₀ in skim milk could be possible which is again significantly ($p < 0.05$) higher than HHP inactivation at the same pressure and time combination. Results revealed that HPCD treatment can be used in combination with pulsed treatment to reduce the pressure level even in a problematic food such as milk.

Table V.7. Inactivation data of two bacteria in skim milk by HPCD at various pressure values, 25 °C for 5 min in comparison with HHP inactivation

	<i>E. coli</i>	<i>L. innocua</i>
HPCD 400 MPa, 5 min	-1.25 ^a	-1.33
HHP 400 MPa, 5 min	-0.52	-0.59
HPCD 400 MPa, 1 min × 5 pulses	-3.66	-3.79
HHP 400 MPa, 1 min × 5 pulses	-2.54	-2.56

^a values are given as log₁₀S(t).

V.4) Conclusions concerning the HHP inactivation of *E. coli* and *L. innocua* in different food media

Results indicate that inactivation of both microorganisms by HHP in kiwifruit juice was higher than the inactivation in pineapple juice which is higher than the inactivation in whole milk i.e., kiwifruit juice > pineapple juice > whole milk. Higher inactivation in fruit juices than in whole milk can be explained by their lower pH values compared to whole milk.

Survival curves of *E. coli* and *L. innocua* between 400 and 600 MPa in whole milk can be successfully described by the Weibull model, preferably by the reduced Weibull model. The logarithm of the time parameter of the reduced Weibull model can be described by the linear function. Hence, a z_p value analogous to the modelling of D value can be defined.

Pulsed pressure treatment could be used instead of continuous HHP at a lower the pressure level to pasteurize (whole) milk. Nevertheless, it should be noted that the total duration of a pulsed HHP treatment at 300 MPa for 2 min \times 10 pulses is 40 min (20 min of holding time + 10 min compression time + 10 min decompression time) which is too long for an industrial application. Therefore an optimization appears necessary between the pulse holding time, number of pulses and pressure levels to reach the desirable number of log-reduction of microorganisms compatible with an industrial application.

HHP treatment at room temperature (20 °C) can also be used to inactivate *E. coli* and *L. innocua* in kiwifruit and pineapple juices at lower pressure values than the ones used in commercial applications (> 400 MPa). However, storage duration and temperature should carefully be optimized to increase the safety of HHP treated fruit juices.

HPCD treatment in combination with pulsed pressure can an efficient way to inactivate the microorganisms in skim milk and to reduce the maximum pressure level for the desired log-reduction.

GENERAL CONCLUSIONS

Compression heating study revealed that ethanol had the highest compression heating values, possibly because of its high value of isothermal compressibility. All the liquid foods (skim milk, whole milk and orange juice) studied were characterized by similar compression heating values as that of water. It was found that, as the initial temperature of the substances increased, the compression heating values also increased. In general, as the pressure level increased the temperature elevation per 100 MPa decreased for ethanol and ethylene glycol; however, the pressure level had no apparent impact on the temperature rise per 100 MPa of the other substances studied. Similarly compression rate had an impact on the compression heating of ethanol and ethylene glycol only.

A second-order polynomial function was proposed to describe the temperature rise as a function of initial temperature and pressure. The fittings indicated that the proposed model could be used successfully to calculate the temperature increase during HHP processing.

Microbial inactivation results indicated that inactivation of both microorganisms by HHP in kiwifruit juice was higher than the inactivation in pineapple juice which is higher than the inactivation in whole milk i.e., kiwifruit juice > pineapple juice > whole milk.

Survival curves of *E. coli* and *L. innocua* between 400 and 600 MPa in whole milk can be successfully described by the Weibull model, preferably by the reduced Weibull model. The logarithm of the time parameter of the reduced Weibull model can be described by the linear function. Hence, a z_p value analogous to the modelling of D value can be defined.

Pulsed pressure treatment could be used instead of continuous HHP at a lower the pressure level to pasteurize (whole) milk. Nevertheless, it should be noted that the total duration of a pulsed HHP treatment at 300 MPa for 2 min \times 10 pulses is 40 min (20 min of holding time + 10 min compression time + 10 min decompression time) which is too long for

an industrial application. Therefore an optimization appears to be necessary between the pulse holding time, number of pulses and pressure levels to reach the desirable number of log-reduction of microorganisms compatible with an industrial application.

HHP treatment at room temperature (20 °C) can also be used to inactivate *E. coli* and *L. innocua* in kiwifruit and pineapple juices at lower pressure values than the ones used in commercial applications (> 400 MPa). However, storage duration and temperature should be carefully optimized to increase the safety of HHP treated fruit juices.

HPCD treatment in combination with pulsed pressure can an efficient way to inactivate the microorganisms in skim milk and to reduce the maximum pressure level for the desired log-reduction.

In the coming years, HHP is likely to be used commercially before the underlying science and its full potential are comprehensively understood. High capital expenditure may limit its application initially but this will be offset by lower operating costs since the energy used to pressurize is less than the energies used in thermal processing and other benefits with respect to product originality. With further progress of technology and its commercialization, it is expected that the cost of the equipment will come down in the near future and the high-pressure processed safe and nutritious products will be available to all consumers at an affordable cost.

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APPENDIX I

List of the publications concerning this thesis

Journal papers

- S. **Buzrul**, H. Alpas, A. Largeteau, G. Demazeau, (2007). Mathematical expression of the temperature profile and experimental determination of compression heating of ethylene glycol during high hydrostatic pressure processing. *High Pressure Research*, 27(1): 93-99.
- S. **Buzrul**, H. Alpas, A. Largeteau, F. Bozoglu, G. Demazeau, (2008). Compression heating of selected pressure transmitting fluids and liquid foods during high hydrostatic pressure treatment. *Journal of Food Engineering*, 85(3): 466-472.
- S. **Buzrul**, H. Alpas, A. Largeteau, G. Demazeau, (2008). Modeling high pressure inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *European Food Research and Technology*, doi:10.1007/s00217-007-0740-7.
- S. **Buzrul**, H. Alpas, A. Largeteau, G. Demazeau, (2008). Pulsed pressure treatment for inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *Journal of Physics: Conference Series*, in print.
- S. **Buzrul**, H. Alpas, A. Largeteau, G. Demazeau, (2008). Inactivation of *Escherichia coli* and *Listeria innocua* in kiwifruit and pineapple juices by high hydrostatic pressure. *International Journal of Food Microbiology*, 10.1016/j.ijfoodmicro.2008.03.015.
- S. **Buzrul**, H. Alpas, A. Largeteau, G. Demazeau, (2008). Efficiency of pulse pressure treatment for inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *Food Microbiology*, submitted.

Poster contributions

S. Buzrul, H. Alpas, A. Largeteau, G. Demazeau, (2007). Pressure inactivation kinetics of *Escherichia coli* and *Listeria innocua* in whole milk. *Joint 21th AIRAPT & 45th EHPRG International Conference*. Catania, Italy.

S. Buzrul, H. Alpas, A. Largeteau, F. Bozoglu, G. Demazeau, (2007). Compression heating of selected pressure transmitting fluids and liquid foods during high hydrostatic pressure treatment. *Joint 21th AIRAPT & 45th EHPRG International Conference*. Catania, Italy.

APPENDIX II

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