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Navin K. Rastogi

# Recent Developments in High Pressure Processing of Foods

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# Recent Developments in High Pressure Processing of Foods

Foreword by Dietrich Knorr

 Springer

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# Foreword

The initiation of the “IFT Non-thermal Division” and the “European Federation of Food Science & Technology (EFFoST) Appropriate Technology Special Interest Group” resulted from a breakfast meeting organized by Dan Farkas at the Annual Meeting of the Institute of Food Technologist (IFT). In that meeting, a number of research projects supported on the national and European level were discussed and there I laid the foundation for the publication of the International Journal in the caption of “Innovative Food Science and Emerging Technologies”. Ever since, this emerging technology has been in explicit use in almost all areas of food processing,. It has attracted the attention of scientists throughout the World, leading to application in different areas, also revealing certain challenging issues of this immanent technology.

The growing interest and significance of high-pressure technology has led to a vast number of papers and scholars and researchers have had to spend quite some effort to identify and sort through these references. Hence, a compilation and review of these references has been in urgent demand. Early in 2007, Navin K. Rastogi published a seminal and widely cited review article in *Critical Reviews in Food Science and Nutrition* (Vol. 47, pp. 69–112). Dr. Rastogi’s present book encompasses all recent and relevant research articles published in the domain of high-pressure technology in food processing with regard to fruits and vegetables, dairy and animal products and serves as a compendium for the food processing industry – and will be a valuable guide for years to come.

I congratulate the author for his achievement in making this information available. It aptly fulfills the objectives of *Springer Briefs* in providing the relevant state-of-the art information in a concise and comprehensive manner.

Berlin University of Technology, Germany

Dietrich Knorr



# Preface

The food industry has long attempted to find adequate means for transforming agricultural produce into a suitable form for human consumption with enhanced safety and marginal cost of production. Growing demands for quality foods including enhanced nutritive value and safety poses great challenges for research. At the same time, consumer expectations need to be met regarding convenience, variety, enhanced shelf life, lower production costs as well as adequate caloric content. Also, the production process should not cause an adverse impact on the environment and should be energy efficient. This calls for advancement and modification of existing procedures, including possible replacements of current techniques applied in the food processing industry. High-pressure technology in food processing is a viable solution for meeting all these requirements. It allows for food preservation by nonthermal means inactivating harmful pathogens and preventing vegetative spoilage by microorganisms by using pressure rather than heat to effect pasteurization. High-pressure technology can lead to adequate food preservation while causing minimal changes regarding taste, texture, appearance, and nutritional value. This technology is among the most prominent recent innovations in food processing.

The present book contains major sections on the application of high pressure on plant products, dairy products, and animal products. While not being able to cover the entire field, the writing of this book was driven by the desire to compile and review the latest literature. I chose to address not only the most recent applications, but also novel and standard applications that have emerged as a response to the challenges faced by the food industry. This book attempts to provide an in-depth view into this important technology and to direct its readers to additional and valuable sources of further information regarding research and development.

My wholehearted thanks go to all members of my family for their support and encouragement during the entire process of writing this book. I sincerely want to thank my project coordinator at Springer, Rita Beck, for her efficient and helpful support.

Mysore, India

Navin K. Rastogi





## About the Author



Dr. Navin K. Rastogi is currently the senior principal scientist in the Department of Food Engineering at the Central Food Technological Research Institute (CFTRI), Mysore, India. His main research interests are in nonthermal processing (high-pressure processing, pulsed electric field, ozone processing), osmotic drying of foods and biomaterials, extraction using liquid membranes, downstream processing of biomolecules, membrane processing, as well as development of various kinds of food products. He has so far published more than 120 papers in refereed international

journals. He is an author/coauthor of 16 book chapters and a co-editor of a book on membrane processing. He has also served as an editor of an archival journal, *Journal of Food Science & Technology* (published by Springer), and has been on the editorial boards of *Journal of Food Engineering* (Elsevier), *Journal of Engineering* (Hindawi), *The Scientific World Journal* (Hindawi), *Research & Reviews: Journal of Food Science & Technology* (STM).

Among the prestigious awards bestowed upon him is the Young Scientist Awards by the Council of Scientific and Industrial Research, Ministry of Science and Technology, New Delhi and Association of Food Scientist and Technologist, India. He was also awarded the Seligman APV fellowship in food engineering, sponsored by the Society of Chemical Industry, London, *Deutscher Akademischer Austauschdienst* (DAAD) Fellowship, Germany and Department of Biotechnology Overseas Fellowship by the Ministry of Science & Technology, New Delhi, India. During these fellowships he worked on high-pressure processing of foods at the University of Reading, UK (with Prof. K. Niranjana), Technische Universität, Berlin,

Germany (with Prof. Dietrich Knorr) and at Ohio State University, U.S.A. (with Prof. V.M. Balasubramaniam). After completing his Bachelor of Science (B.Sc.) at Lucknow University, he obtained a further Bachelor of Technology (B.Tech.) from Harcourt Butler Technology Institute, Kanpur followed by a Master of Business Administration (M.B.A.) and a Master in Engineering Management (M.E.M.) from Rohilkhand University, Bareilly and Sri Jayachamraja College of Engineering, Mysore, respectively. Thereafter, Dr. Rastogi did his doctoral work in food engineering at CFTRI and the resulting Ph.D. degree was awarded by the University of Mysore, India.

# Abstract

Fruit processing and preservation technologies must attempt to retain fresh-like characteristics while providing an acceptable and convenient shelf life as well as assuring safety and nutritional value. Processing technologies include a wide range of methodologies to inactivate microorganisms, improve quality and stability, and preserve and minimize changes of fruit fresh-like characteristics. Destruction of microorganisms, inactivation of enzymes at low or moderate temperatures without changing organoleptic and nutritional properties, as well as recent commercial success stories show that high-pressure technology has a great potential, which can be used for the development of diversified value-added food products with exciting opportunities for industry such as products with novel physicochemical and sensory properties. In recent years there has been a significant increase in the number of scientific papers in literature demonstrating novel and diversified uses of this technology – thus this can be considered as a new, prominent emerging technology. The book reviews the effects of high pressure on the quality and safety of plant, dairy, and animal foods. Selected practical examples involving the use of high-pressure processing and benefits derived from this technology in the food industry are comprehensively presented and discussed.



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# Chapter 1

## Introduction

“Necessity is the mother of invention”. This adage aptly applies regarding nonthermal processing techniques in the food processing sector. There is a growing consumer interest for fresh and mildly processed foods with preserved nutritional and organoleptic characteristics that can be stored in the refrigerator for extended periods of time without compromising safety and can be quickly heated before consumption. Increasing consumer demand for high-quality convenient food products with natural flavor and taste, free from additives and preservatives have posed greater challenges to food scientists and technologists. Novel and alternative food processing methods, as well as combinations of existing methods, are continually being sought by the food industry in pursuit of producing better quality foods in an efficient and economical manner. These innovative processing methods involve environmentally friendly and sustainable food manufacturing techniques, which call for low energy and reduced water requirement. Hence, new innovations, technologies, and concepts continue to emerge to process foods with maximum safety and quality without the disadvantages of conventional processing. Quality and safety of food products are the two factors that mostly influence the choices made by today’s increasingly demanding consumers. Emerging nonthermal processes such as application of high pressure, pulsed electric field, ultrasound, cold plasma, and ultraviolet light are such alternatives for processing foods with maximum safety and quality. Taking advantage of specific potentials and opportunities of these new processes, including the understanding and control of the complex process-structure-function relationships, offers the possibility for a science-based development of tailor-made foods (Knorr et al. 2011). In recent years, there has been a significant increase in the number of scientific papers demonstrating novel and diversified uses of these technologies.

High-pressure processing (HPP) has gained momentum as a processing technique that aids in retention of fresh appearance in foods. It holds promise as a method to process premium value food products while retaining quality attributes. It is an emerging food treatment that makes food safer and extends its shelf life, while allowing the food to retain many of its original qualities and healthy attributes. It retains food quality, maintains natural freshness, and extends microbiological

shelf life. It can improve food safety by destroying the bacteria that cause food-borne illness and spoilage, and parasites that cause diseases. It also leads to the specific product quality and addresses productivity issues without the use of flavor-altering additives or methods. Unlike the conventional heat treatments, it is a nonthermal preservation and pasteurization technique, which results in little or no change in the organoleptic and nutritional attributes of the product. High-pressure-processed foods have better texture, fresher taste, and improved appearance and increased retention of nutrients in comparison to thermal-processed foods. It has been regarded as one of the best innovations in food processing in 50 years (Dunne 2005).

Over the past decades, high-pressure processing has increasingly being acknowledged as unit operation in food processing and preservation. In current commercial applications, high pressure is essentially a ‘nonthermal’ pasteurization process. Since the process involves minimal heating, many quality aspects can be remarkably similar to those of the unprocessed counterpart (Jolie et al. 2012). High-pressure processing was recognized as one of the most emerging food-processing technologies that destroy food-borne pathogens and spoilage organisms, thereby ensuring product safety and enabling longer shelf life. Apart from that it can reliably inactivate enzymes, germinate and inactivate bacterial spores, marinate meats, shuck oysters, extend shelf life, reduce the potential for food-borne illness, pressure-shift freezing or thawing, promote ripening of cheeses, minimize oxidative browning etc.. High-pressure processing inactivates microbes by damaging their outer cell membrane and essential proteins in cells. Furthermore, it also inactivates some food quality related endogenous enzymes present in foods due to its influence on the unfolding of protein chains. In contrast to heat treatment, high pressure affects only noncovalent bonds (hydrogen, ionic, and hydrophobic bonds) and has little effect on chemical constituents associated with desirable food qualities such as taste, flavor, color, or nutritional content and, therefore, the processed products stay quite close to a ‘fresh-like’ product (Hayashi 1990; Balci and Wilbey 1999).

High-pressure treatment (HPT) is based on two fundamental principles: the Le Chatelier principle, which proposes that pressure favors all structural reactions and changes that involve a decrease in volume; and the isostatic principle, which proposes that the distribution of pressure is proportional in all parts of a foodstuff irrespective of its shape and size (Fragoso et al. 2011; Barba et al. 2012). It is defined as a method of food preservation that involves subjecting food to a high hydrostatic pressure (300–700 MPa), with or without the addition of heat, to achieve microbial inactivation while achieving the consumer-desired qualities, i.e., retention of freshness and nutritive value of food products. From a nutritional prospective it is apparent that high-pressure processing is an excellent food processing technology which has the potential to retain compounds with health benefits in foods. Therefore, high-pressure processed foods could be sold at a premium over their thermally processed counterparts. Pressures used are almost ten times greater than in the deepest oceans on Earth. The technology has been adopted and adapted by the food industry from the materials and process-engineering industries where it has been commercially used in sheet metal forming and isostatic pressing of advanced materials such as turbine components and ceramics.

The era of high-pressure processing of foods commenced with the pioneering work of Hite (1899) for preservation of milk, and the scope of this technology was broadened for processing and preservation of fruits and vegetables (Hite et al. 1914). In 1990, the potential of the technology was demonstrated by preserving acidified shelf-stable food. However, the high cost of high-pressure equipment has been responsible for the late commercial success of this technology (Gould 1995; Galazka and Ledward 1995; Balci and Wilbey 1999). The ability of high pressure to inactivate microorganisms and spoilage-catalyzing enzymes, whilst retaining other quality attributes, has encouraged Japanese and American food companies to introduce high-pressure processed foods in the market (Mermelstein 1997; Hendrickx et al. 1998). The first high-pressure processed foods were introduced to the Japanese market in 1990 by Meidi-ya, who have been marketing a line of jams, jellies, and sauces packaged and processed without application of heat (Thakur and Nelson 1998). Later, M/s Fresherized Foods, Texas, US introduced high-pressure preserved guacamole dip. Its potential is now being realized in the commercial market and in the future may lead to a wide variety of shelf-stable diversified food products, which cater to the consumer with a variety of tastes and nutritive advantages. Currently, there are a number of successful high-pressure processed products on global food markets including sliced small goods in Spain and the U.S., fruit juices, jams, jellies, rice cakes, and raw squids in Japan, fruit juices, especially apple and orange juice, in France, Italy, Portugal, the U.K. and the U.S., salsa, guacamole meal kits, oysters in their shells, ready-to-eat meats in the U.S., and apple sauce in Canada (Hugas et al. 2002).

These new dimensions of food processing give food processors the opportunity to process foods with cleaner ingredients and fewer additives and in some cases improving texture (Balasubramaniam and Farkas 2008). Biological effects of high pressure such as inactivation of microorganisms or changing functional properties of food biopolymers, have been known for decades, but only in the last few years foods preserved by high pressure became commercial reality. Today, high-pressure technology is acknowledged to have the promise of producing a wide range of products, whilst simultaneously showing potential for creating a new generation of value-added foods. In general, high-pressure technology can supplement conventional thermal processing for reducing microbial load, or substitute the use of chemical preservatives (Rastogi et al. 1994). Over the past two decades, this technology has attracted considerable research attention, mainly relating to the extension of keeping quality (Cheftel 1995; Farkas and Hoover 2001), changing the physical and functional properties of food systems (Cheftel 1992), and exploiting the anomalous phase transitions of water under extreme pressures, e.g., lowering of freezing point with increasing pressures (Kalichevsky et al. 1995; Knorr et al. 1998).

High pressure hardly effects small molecules such as flavor compounds, vitamins, and pigments compared to thermal processes. Hence, the quality of pressure-pasteurized food is very similar to that of fresh food product. High pressure also provides a unique opportunity to create and control novel food textures in biopolymers such as proteins and polysaccharides. The changes may produce new textures that may facilitate the development of new and novel products (Hayashi 1990).

A typical HPP system consists of a high-pressure vessel, pressure generation system, temperature and pressure monitoring systems, as well as a material handling system. Hydrostatic pressure is applied to food products by pumping pressure-transferring medium (usually water) that surrounds the product. The pressure is transmitted to food products equally from all sides. This equal distribution of pressure is the reason why foods are not crushed during treatment. It can be conducted at ambient or refrigerated temperatures, thereby eliminating thermally induced cooked off-flavors. The technology is more useful for heat-sensitive products. The product packaging must be able to withstand a change in volume up to 15 %, followed by a return to its original size. The application of high pressure increases the temperature of liquid components in food by approximately 3 °C per 100 MPa. If the food contains a significant amount of fat, such as butter or cream, the temperature rise is greater (8–9 °C/100 MPa) (Rasanayagam et al. 2003). Foods cool down to their original temperature on decompression if no heat is lost to or gained from the walls of the pressure vessel during the holding stage.

Both liquid and solid foods can be processed using high pressure. The foods with a high acid content (low pH foods) are the best candidates for HPP. It cannot be used to make shelf-stable low-acid products such as vegetables, milk, or soups because of the inability of this process to destroy spores. But, it can be used to extend the refrigerated shelf life of these products and to eliminate the risk of various food-borne pathogens. If the food does not contain enough moisture, then HPP may not be effective for microbial destruction. Food materials containing entrapped air such as strawberries or marshmallows would be crushed under high-pressure treatment.

High-pressure processing can be applied to a range of foods, including juices and beverages, fruits and vegetables, meat-based products (cooked and dry ham, etc.), fish, and precooked dishes, with meat and vegetables being the most popular applications (Norton and Sun 2008). The application of high pressure in food technology is a useful tool to combine a minimal processing that increases the shelf life of food products, maintaining their safety and nutritional properties and with minimal changes in flavor or taste. The commercially available food products that are processed by high pressure are presented in Table 1.1. With a steadily increasing number of commercial applications being introduced in the market, high-pressure pasteurization is growing out of its infancy. The product range is increasing and spreading from its origins in Japan, followed by the United States and now Europe (Hogan et al. 2005). Several companies offering a wide variety of high-pressure equipment to the food industry are listed in Table 1.2.

Depending on the operating parameters and the scale of operation, the cost of high-pressure treatment is typically around US\$ 0.05–0.5 per liter or kilogram, the lower value being comparable to the cost of thermal processing (Thakur and Nelson 1998; Balasubramaniam 2003). For both the pasteurization and sterilization processes, a combined treatment of high pressure and temperature are frequently considered to be most appropriate (Farr 1990; Patterson et al. 1995). At high pressures, microbial death is considered to be due to permeabilization of cell membranes. At a pressure of about 400 MPa, the structure and cytoplasmic organelles of *Saccharomyces cerevisiae* were grossly deformed leading to the leakage of large

**Table 1.1** Commercially available high-pressure processed food products available worldwide

Product	Name of the company
<i>Fruits and vegetable products</i>	
Orange juice	M/s Ultifruit, France
Mandarin juice	M/s Wakayama Food Industries, Japan
Fruit juices	M/s Pampryl, France
Fruit and vegetable juices	M/s Odwalla, U.S.
Apple juice	M/s Frubaca, Portugal
Fruit juices and smoothies	M/s Orchard House, UK
Jams, fruit sauces, yogurt and jelly	M/s Meida-Ya, Japan
Fruit jams	M/s Solofruita, Italy
Tropical fruits	M/s Nishin Oil Mills, Japan
Guacamole, salsa dips, ready meals, and fruit juices	M/s Avomex, U.S.
<i>Animal products</i>	
Beef	M/s Fuji Ciku Mutterham, Japan
Hummus	M/s Hannah International, U.S.
Ham	M/s Hormel Foods, U.S.
Poultry products	M/s Purdue Farms, U.S.
Oysters	M/s Motivait Seafoods, U.S.; Goose Point Oysters, U.S. Joey Oysters, U.S.
Sliced ham and tapas	M/s España, Spain

From Hogan, E., Kelly, A.L., and Sun, D.W. 2005. *Emerging Technologies for Food Processing*, ed. D.W. Sun, 4–30. London: Elsevier Ltd. With permission

**Table 1.2** Main suppliers of high-pressure processing equipment and services

Name of the company	Specialization
M/s Resato International <a href="http://www.resato.com">http://www.resato.com</a>	Manufactures laboratory and industrial machines Pressure shift freezing systems Reciprocating intensifiers suitable for one or multiple autoclave systems (up to 1,400 MPa)
M/s Avure Technologies Inc., <a href="http://www.avure.com">http://www.avure.com</a>	Manufactures batch presses that pasteurize prepared ready-to-eat foods Unique pumping systems to enhance throughput (600 MPa)
M/s Elmhurst Research, Inc., <a href="http://www.elmhurstresearch.com">http://www.elmhurstresearch.com</a>	Designs and manufactures batch presses Patented vessel technology developed exclusively for food processing industry (689 MPa)
M/s Engineered Pressure Systems Inc. <a href="http://www.epsi-highpressure.com">http://www.epsi-highpressure.com</a>	Manufactures laboratory and industrial equipment Manufacture hot, cold, and warm isostatic presses (100–900 MPa)
M/s Kobelco, <a href="http://www.kobelco.co.jp">http://www.kobelco.co.jp</a>	Manufactures laboratory and industrial equipment Manufacture hot and cold isostatic presses (98–686 MPa)
M/s Mitsubishi Heavy Industries, <a href="http://www.mhi.co.jp">http://www.mhi.co.jp</a>	Manufactures laboratory and industrial equipment Manufactures isostatic pressing system with large operating temperature range as option (686 MPa)

(continued)

**Table 1.2** (continued)

Name of the company	Specialization
M/s NC Hyperbaric, <a href="http://www.nchyperbaric.com">http://www.nchyperbaric.com</a>	Manufactures industrial equipment Designed a system to work with different volumes (600 MPa)
M/s Stansted Fluid Power Ltd. <a href="http://www.sfp-4-hp.demon.co.uk">http://www.sfp-4-hp.demon.co.uk</a>	Manufactures equipment for R&D and industrial scale equipment Single and multiple vessels with wide temperature (up to 1,400 MPa)
M/s Uhde Hockdrucktechnik, <a href="http://www.uhde-hpt.com">http://www.uhde-hpt.com</a>	Manufactures equipment for industry and research purposes Develops plant processes from initial testing to full-scale application (700 MPa)

Pressure capacity of standard machines is provided in parentheses (From Norton, T., and Sun, D.W. 2008. *Food Bioprocess Technol.* 1: 2–34. With permission)

quantities of intracellular material, whilst at 500 MPa the nucleus could not be recognized and intracellular material was completely lost (Farr 1990; Osumi et al. 1996). Bacterial spores require much higher pressures (>1,200 MPa) to become inactivated (Knorr 1995). The initiation of germination or inhibition of germinated bacterial spores and inactivation of piezo-resistive microorganisms can be achieved in combination with moderate heating or other pretreatments such as ultrasound. Process temperature in the range 90–121 °C in conjunction with pressures of 500–800 MPa have been used to inactivate spore-forming bacteria such as *Clostridium botulinum*. Thus, sterilization of low-acid foods (pH>4.6), will most probably depend on a combination of high pressure with other treatments.

The main advantages of this technology can be summarized as:

1. Uniform and instantaneous distribution of pressure irrespective of size and geometry
2. Effectiveness at ambient temperature or even lower temperatures
3. Elimination of thermal damage and no use of chemical preservatives/additives
4. Lower operating cost and less process times
5. Performance on packed foods, so cost-intensive aseptic sterilization can be avoided
6. Higher retention of texture, appearance, natural color, flavor, and health-promoting substances compared to thermal processing
7. High retention of nutritional values, freshness, and flavor
8. Environment friendliness

The enormous availability of literature substantiates this technology as a prominently emerging technology. From a nutritional perspective, high-pressure processing is an attractive food preservation technology. The capabilities and limitations of high-pressure processing have been extensively reviewed (Thakur and Nelson 1998; Smelt 1998; Cheftel 1995; Knorr 1995; Farr 1990; Tiwari et al. 1999; Cheftel et al. 2000; Messens et al. 1997; Otero and Sanz 2000; Hugas et al. 2002; Lakshmanan

et al. 2003; Balasubramaniam 2003; Matser et al. 2004; Hogan et al. 2005; Mor-Mur and Yuste 2005; Rastogi et al. 2007; Rastogi 2009a, b, 2010; Campus 2010). Many of the early reviews primarily focused on the microbial efficacy or general aspects of high-pressure processing. This review comprehensively discusses the effect of high pressure on color, flavor, texture, nutrition, and sensory characteristics. Selected applications of this technology in fruits and vegetable, dairy and animal product-processing industries have also been covered.

## Chapter 2

# High-Pressure Processing of Plant Products

HPT of plant products is gaining popularity in the food industry because of its ability to inactivate microorganisms and some enzymes near room temperature with little impact on flavor or nutritional attributes of the food.

### 2.1 Fruits

#### 2.1.1 Apples

Apple cubes (var. Granny Smith and Pink Lady) in pineapple juice subject to HPT (600 MPa) resulted in no visible color change during 4 weeks of storage at 4 °C. The treatment significantly reduced residual PPO activity, while PME activity was not affected. Pineapple juice in combination with high pressure can be used as a preservation method for minimally processed apples (Perera et al. 2010). HPT (200–650 MPa) of apple cubes in acidified glucose solution (25.0 %) resulted in 6-log reduction of *Candida lipolytica* and *Escherichia coli* at 400 and 600 MPa, respectively. The microbiological shelf life of the product was extended from 15 days to 90 day during refrigerated storage (7 °C). The treatment had no significant effect on the hardness of the apple pieces, but addition of sodium metabisulfite helped to prevent browning during the entire storage period (Vercammen et al. 2012).

HPT (150 MPa) in presence of argon gas resulted in delayed browning and microbial growth as well as lower respiration rate, ethylene production, and total phenolics of fresh-cut apples in comparison with controls, when stored at 4 °C for 2 weeks. Dipping the sample into 0.5 %w/w ascorbic acid, 0.5 %w/w citric acid, and 0.5 %w/w calcium chloride for 5 min reduced the changes in color and firmness of apple wedges during high-pressure treatment and retained good sensory attributes (Wu et al. 2012).

Application of high pressure (500 MPa) preserves and even improves the availability of minerals and antioxidants. The bioaccessibility of calcium, iron, and zinc



was increased by 2.11–303 %, 4.63–10.93 %, and 8.68–28.93 %, respectively. At the same time, the dialyzability and solubility of these minerals was reduced and antioxidant activity was increased (Labarca et al. 2011). Husband et al. (2011); indicated that combining high pressure and thermal processing effectively reduces the allergenicity of apples. Kim et al. (2012) demonstrated that treatment of apple juice at 500 MPa, 25 °C, 3 min did not cause significant changes in vitamin C content, whereas total polyphenol content was increased. The product was microbiologically safe without physicochemical changes during 21 days of storage at 4 °C. Novotna et al. (1999) showed that the aroma of apple juice subjected to HPP was superior to that of pasteurized juice.

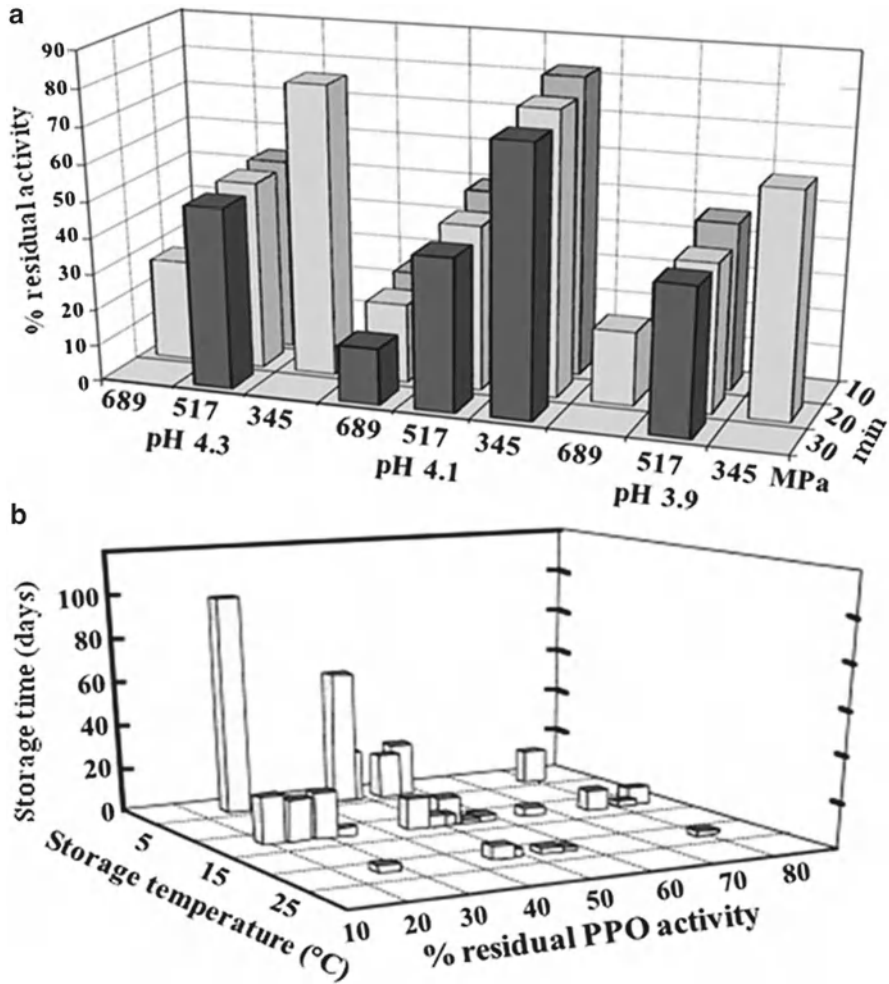
Buckow et al. (2009) developed a polynomial model to describe the rate of PPO inactivation in cloudy apple juice as a function of pressure and temperature and showed synergistic effects of pressure and temperature on the inactivation of apple PPO at pressures above 300 MPa and antagonistic effects at lower pressures. Landl et al. (2010) demonstrated that HPP (400 MPa) of apple puree resulted in no significant changes in total vitamin C and total phenolic content during 3 weeks of storage at 5 °C. However, treatment at 600 MPa led to a decrease in total phenolic content as did pasteurization treatment (75 °C, 10 min). Microbial counts were reduced below the detection limit (50 CFU/g) and storage revealed no further growth.

### **2.1.2 Apricots**

High pressure (300–500 MPa, 5–20 min) applied to apricot nectar resulted in activation of PPO and POD, whereas PME was not changed. High-temperature short-time (HTST, 110 °C, for 8.6 s) treatment induced a complete inactivation of these enzymes. HPT increased total and individual phenolics in apricot nectars compared to HTST-treated apricot nectars. HPT also had no effect on total carotenoids and individual carotenes in apricot nectars except that treatment at 500 MPa, 20 min increased total carotenoids and  $\beta$ -carotene. Moreover, the color of high-pressure-treated apricot nectars was closer to the untreated apricot nectar (Huang et al. 2012).

### **2.1.3 Avocados**

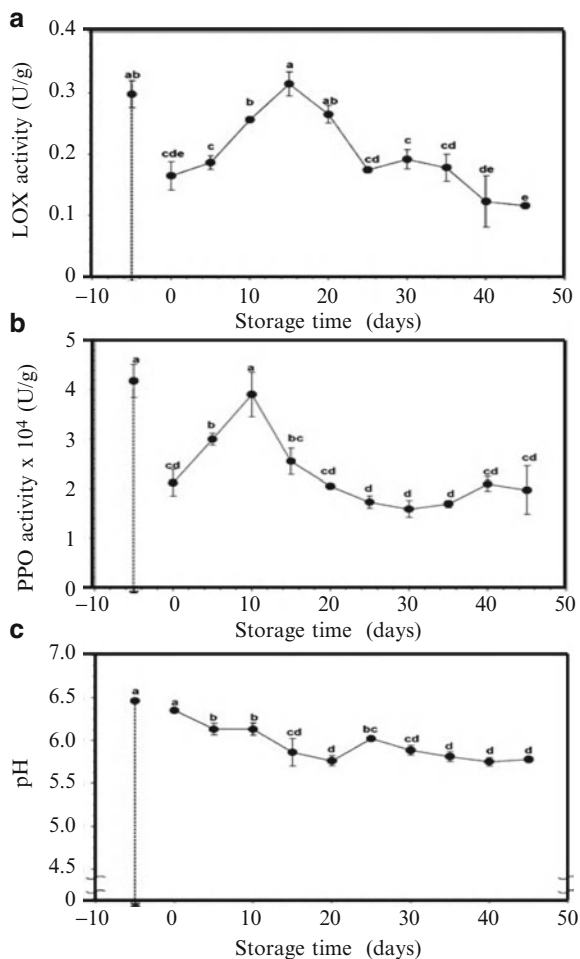
Lopez et al. (1998) pointed out that standard plate as well as yeast and mold counts of high-pressure-treated (345–689 MPa, 710–30 min) avocado puree was less than 10 CFU/g during 100 days of storage at 5 °C, 15 °C, or 25 °C. For treatment at pH 4.1 and 689 MPa, residual PPO activity was reduced to 24.7 %, 21.8 %, and 15.6 % for 10, 20, or 30 min of processing, respectively (Fig. 2.1a). Avocado puree with a residual PPO activity of less than 45 % maintained an acceptable color for 60 days during storage at 5 °C (Fig. 2.1b). Palou et al. (2000) indicated that HPT (689 MPa) resulted in complete inactivation of PPO and lipoxygenase (LOX) and reduction in standard plate count to less than 10 CFU/g without significantly affecting the



**Fig. 2.1** (a) Effect of HPT and initial pH on residual polyphenoloxidase activity of avocado puree; (b) Effect of residual polyphenoloxidase activity and storage temperature on the storage time needed to lose the *green color* component in avocado puree (From Lopez, M.A., Palou, E., Barbosa-Cánovas, G.V., Welti-Chanes, J., and Swanson, B.G. 1998. *Food Res. Intl.* 31: 549–556. With permission)

sensory properties with extended shelf life. Later, Velazquez and Brenes (2010) showed that HPP (600 MPa and 3 min) of avocado paste resulted in a decrease in PPO and LOX activities, which were reactivated and reached the original values during 10–15 days of storage and then started to decline again until the end of the storage period (Fig. 2.2a, b). The pH of the pulp consistently declined during the first 20 days of storage (Fig. 2.2c). Enzyme reactivation, cell disruption, and a gradual migration of intracellular components such as organic acids were proposed as the main mechanisms for the deterioration of high-pressure-treated avocado paste during storage.

**Fig. 2.2** Effect of HPT (600 MPa for 3 min) and storage (4 °C for 45 days) on (a) lipoxygenase (LOX), (b) PPO activity of avocado paste, (c) the pH of avocado paste. (Velazquez, D.A.J. and Brenes, C.H. 2010. *J. Food Sci.* 75: S264–S270. With permission)



Further, Velazquez and Brenes (2012) demonstrated that HPT (600 MPa, 3 min) induced a significant increase in individual carotenoids namely neoxanthin-b,  $\alpha$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin,  $\beta$ -carotene, and lutein concentrations as well as total extractable carotenoids. The carotenoid levels declined during storage (40 days, 4 °C), but at the end of the product's sensory shelf life were higher than those initially present in unprocessed avocado paste.

### 2.1.4 Berries

Terefe et al. (2009, 2010) found that HPT (600 MPa, 60 °C, 10 min) led to substantial inactivation of POD (58 %) in strawberries, whereas no significant reduction in PPO, total polyphenol, and total anthocyanin content was observed. Best quality

retention of strawberry products was obtained when HPP was combined with vacuum packaging in high-barrier packaging material and refrigerated storage. Patras et al. (2009a) demonstrated that HPT (400–600 MPa, 15 min, 10–30 °C) did not yield significant changes in ascorbic acid and anthocyanin content and antioxidant activities in strawberry and blackberry purees. The treatment retained the redness of purees as compared to thermally processed samples. Verbeyst et al. (2010) indicated that HPT (200–700 MPa, 80–130 °C) of strawberry paste resulted in first-order degradation kinetics of anthocyanins (pelargonidin-3-glucoside). At constant pressure, anthocyanin concentration decreased with an increase in treatment time and the degradation was accelerated at higher temperatures. At constant temperature, anthocyanins were more rapidly degraded as the pressure increased, but the effect of increasing pressure was smaller than the effect of increasing temperature. Cao et al. (2011) indicated that HPT (400–600 MPa) effectively retained monomeric anthocyanins, polymeric color and redness, phenolic compounds, and color of strawberry pulps. It partially inactivated food quality-related enzymes such as PPO, POD, and  $\beta$ -glucosidase (Fig. 2.3). Further, Cao et al. (2012) demonstrated that HPT (600 MPa) of cloudy and clear strawberry juices after 6 months of storage at 4 °C resulted in 39.41 %, 29.76 %, and 16.22 % decrease in ascorbic acid, anthocyanins, and total phenols, respectively in cloudy juices, whereas the corresponding values for clear juice were 48.91 %, 7.02 %, and 13.82 %, respectively. The decrease of these indices at 25 °C storage was almost doubled, while total difference color and browning degree were significantly higher.

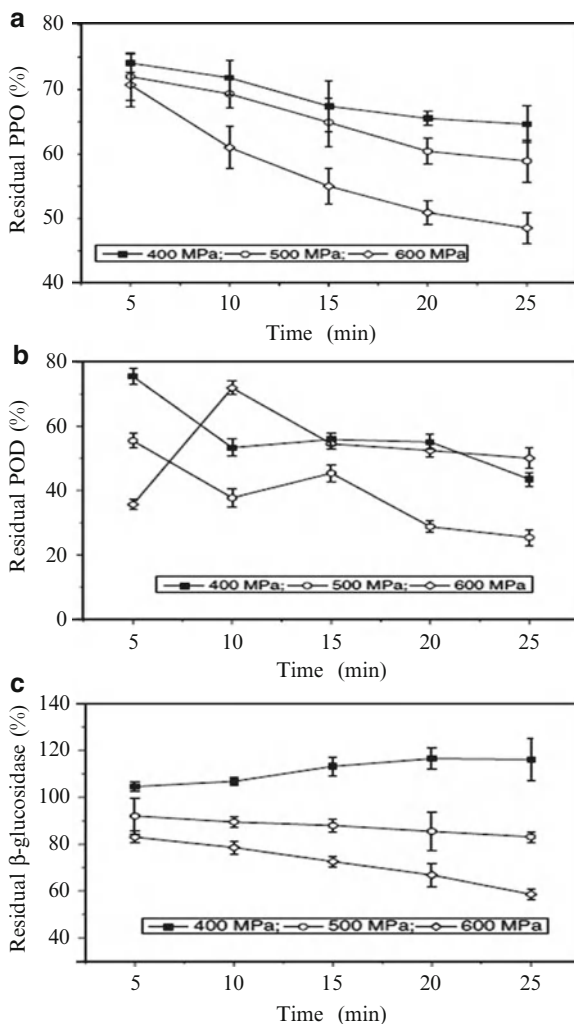
Bodelon et al. (2012) demonstrated that HPT (100–400 MPa, 20 °C and 50 °C, 15 min) resulted in no change in ascorbic acid and concentration of anthocyanins. PME activity decreased in the samples pressurized at 50 °C, but, a gel-network formation in the strawberry puree was not found; however, the latter was observed in the control and in the sample pressurized at 20 °C throughout cold storage. The temperature of processing had a significant effect on the color of strawberry puree, but no significant difference was observed after cold storage.

Lambert et al. (1999) demonstrated that strawberry aroma can be characterized by two main components (nerolidol and furaneol) and no major changes in strawberry aroma profiles were observed up to 500 MPa. But, higher pressure (800 MPa) induced significant changes in the aroma profiles due to the formation of new compounds namely 3,4-dimethoxy-2-methyl-furan and  $\gamma$ -lactone (Fig. 2.4).

Fraeye et al. (2010) studied infusion of PME and calcium prior to thermal, high-pressure, and combined high-pressure/thermal processing of strawberries. Processing of strawberries caused a decrease in firmness, which was reduced by infusion of PME and calcium chloride. PME was able to decrease the degree of methoxylation of pectin, accompanied by an increased cross-linking of the chains.

Hyperbaric storage (25–220 MPa, 15 days) at room temperature (20 °C) reduced the initial microbial load of the strawberry juice by more than 2 log units to levels below the limit of detection. Moreover, pressure was effective to attenuate viscosity and color losses in the samples stored at 20 °C. Stability of the samples after the hyperbaric storage was good when the samples were kept under refrigeration at atmospheric pressure for 15 additional days (Bravo et al. 2012).

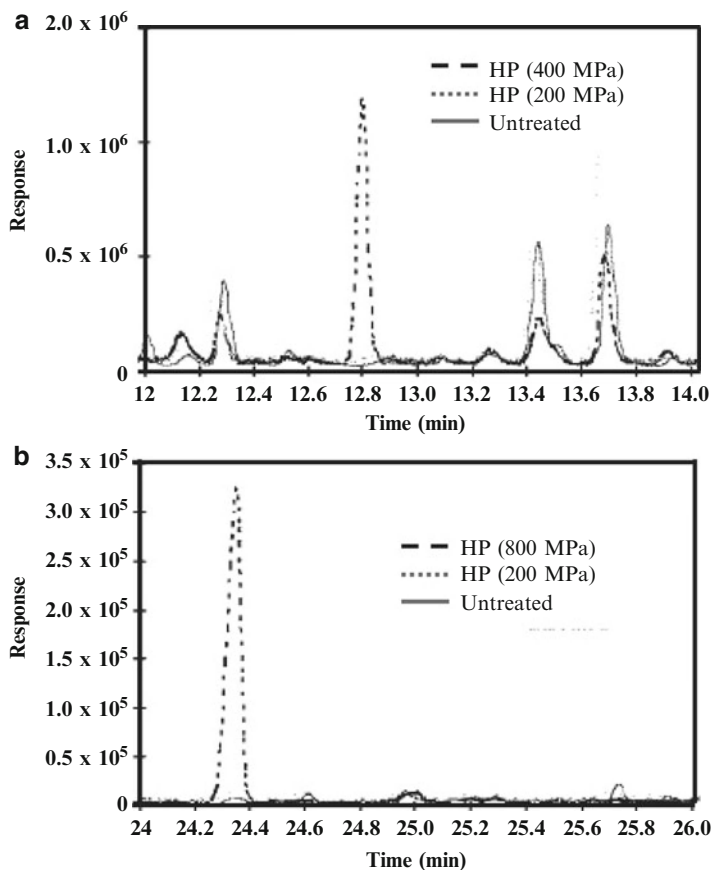
**Fig. 2.3** The effects of high hydrostatic pressure on (a) PPO, (b) POD, and (c)  $\beta$ -glucosidase activity in strawberry pulps (From Cao, X., Yan, Z., Fusheng, Z., Yongtao, W., Jianyong, Y., Xiaojun, L. 2011. *J. Sci. Food Agric.*, 91(5): 877–885. With permission)



Verbeyst et al. (2011) studied the combined effect of high temperature and high pressure on the degradation of cyanidin-3-glycosides in raspberries. Anthocyanin degradation was found to increase with increasing temperature as well as with increasing pressure. Cyanidin-3-glucorutinoside showed less degradation in comparison to the other cyanidins. Cyanidin-3-rutinoside experienced the smallest effect of temperature and the strongest effect of pressure.

### 2.1.5 Cashew Apples

HPT (350–400 MPa, 3 or 7 min) of cashew-apple juice reduced the aerobic mesophilic bacteria count to a level below the detection limit as well as resulted in



**Fig. 2.4** Volatile compounds appearing after HPT (800 MPa, 20 min, 20 °C). Peaks are characterized by retention time of (a) 12.8 min attributed to 3,4-dimethoxy-2-methylfuran or 2-5-dimethyl-4-methoxyfuran-3, (b) 24.3 min attributed to a lactone volatile compound (From Lambert, Y., Demazeau, G., Largeteau, A., and Bouvier, J. M. 1999. *Food Chem.* 67: 7–16. With permission)

complete inactivation of yeast and filamentous fungi. The treated juice was stored for 8 weeks at 4 °C without any significant change in product quality (Lavinias et al. 2008).

### 2.1.6 Grapefruit

Naringin (a flavanone glycoside) is the dominant bitter principle in grapefruit juice. Application of high pressure was shown to enhance the reduction of naringin to naringenin (a tasteless compound) using naringinase immobilized on calcium alginate. Under atmospheric pressure, naringin reduction was only 35 % in a model system,

but it was found to be 75 % under high pressure (160 MPa, 37 °C, 20 min, Ferreira et al. 2008). Further, optimized conditions indicated that use of high pressure (205 MPa, 60 °C, 30 min) resulted in 81 % naringin reduction (Ribeiro et al. 2010).

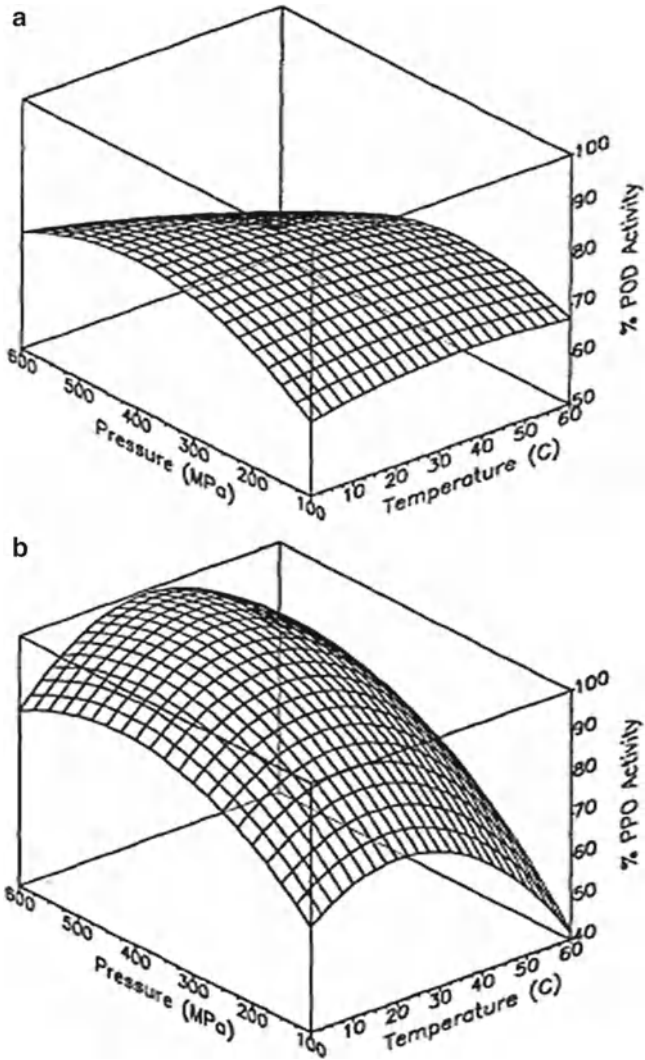
Uckoo et al. (2012) demonstrated that HPT provided 'fresh-like' grapefruit juice without altering the levels of beneficial bioactive compounds. The retention of ascorbic acid was found to be significantly higher in HPP samples as compared to thermally treated samples. Further, these levels were gradually decreased during 0 and 7 days of storage. The levels of citric acid, flavonoids, limonoids, and furocoumarins in HPP samples did not change significantly, whereas the level of carotenoids was lower for both the treatments as well as for the control after 21 days of storage.

### 2.1.7 Grapes

Moio et al. (1994) demonstrated that HPT (500 MPa for 3 min) sterilized white grape 'must' with little changes in physicochemical properties, whereas red grape 'must' did not show higher stability due to the natural microflora present in it. Rastogi et al. (1999) studied the combined effects of HPT (100–600 MPa, 0–60 °C) on inactivation of endogenous enzymes in order to develop shelf-stable red grape juice. The lowest POD (55.75 %) and PPO (41.86 %) activities were found at 60 °C, with pressure at 600 and 100 MPa, respectively (Fig. 2.5). Corrales et al. (2008a, b) indicated that HPT (600 MPa) of red grape skins resulted in increased extraction of total phenolic content, which led to threefold higher antioxidant capacity along with the higher extraction of acylated anthocyanins. The maximum antioxidant capacity was achieved when the extraction was carried out at 70 °C, using 50 % ethanol concentration and pressures up to 600 MPa. In addition, the antioxidant capacity of the extracts increased with extraction time (Fig. 2.6). The highest levels of total anthocyanin monoglucosides were obtained at pressures of 200 MPa, whereas pressures of 600 MPa were optimal for the extraction of acylglucosides (Corrales et al. 2009). Addition of partially purified copigments such as rosemary and thyme polyphenolic extracts with muscadine grape juice during HPT (400 and 550 MPa, 15 min) increased color and antioxidant activity and reduced phytochemical losses during subsequent storage (Pozo et al. 2007). Casazza et al. (2012) demonstrated that use of high-pressure/high-temperature for the extraction resulted in higher total polyphenol and total flavonoid yield from grape skins. Chauhan et al. (2011) pointed out that the maximum retention of total antioxidant activity, phenolics, and flavonoids in the black grape juice were found at optimum level (550 MPa, 44 °C, 2 min).

### 2.1.8 Guavas

HPT (600 MPa, 15 min) of guava puree completely killed microbes and partially inactivated enzymes; the puree was stored up to 40 days at 4 °C without any

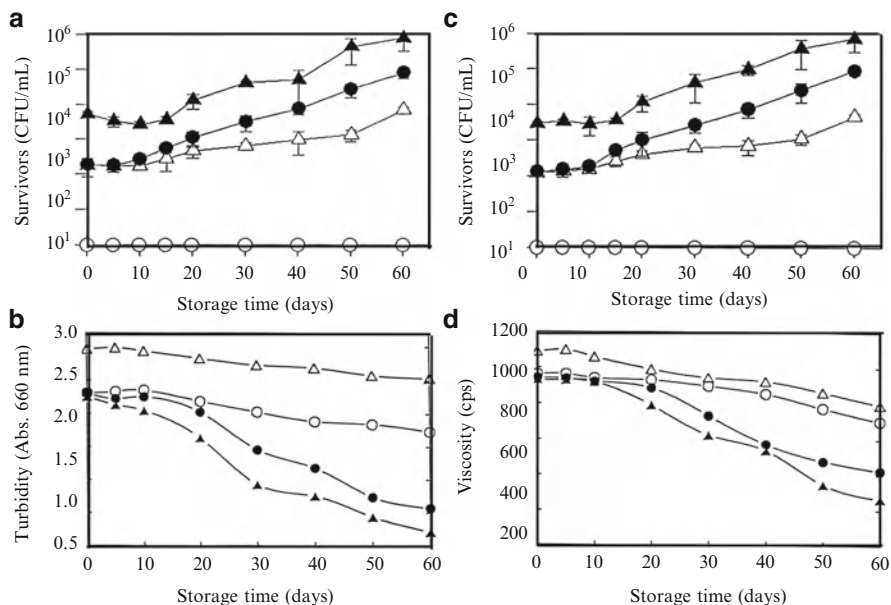
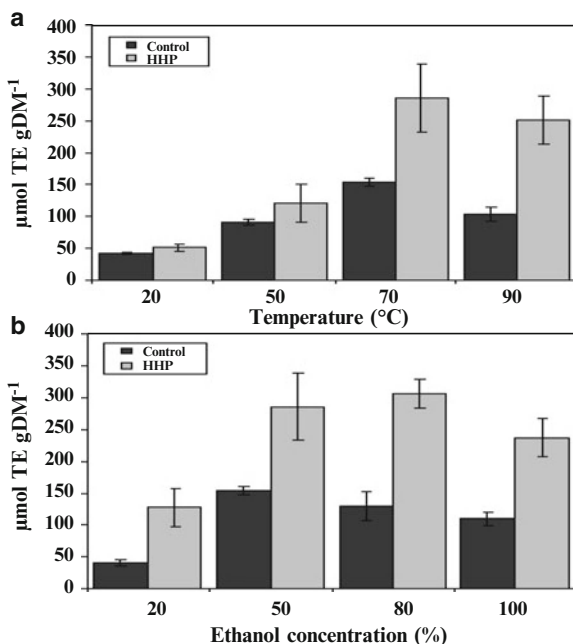


**Fig. 2.5** Response surfaces showing effect of pressure and temperature on (a) % POD activity, (b) % PPO activity (From Rastogi, N.K., Eshtiaghi, M.N., and Knorr, D. 1999. *Food Biotechnol.* 13: 195–208. With permission)

change in color, cloudiness, ascorbic acid content, flavor distribution, and viscosity (Fig. 2.7, Gow and Hsin 1996). The treatment resulted in an increase in the levels of methanol, ethanol, and 2-ethylfuran throughout the entire storage period (Gow and Hsin 1999).



**Fig. 2.6** (a) Effect of high pressure (600 MPa) at (a) different temperatures, (b) ethanol concentrations on the antioxidant capacity of Dornfelder *Vitis vinifera* L. grape skin extracts expressed as  $\mu\text{mol TE/gDM}$  (Corrales, M., Fernandez, G.A., Butz, P., and Tauscher, B. 2009. *J. Food Eng.* 90: 415–421. With permission)



**Fig. 2.7** Effects of high pressure treatment and thermal pasteurization on the changes in (a) total plate counts, (b) turbidity, (c) yeast and mould counts, and (d) viscosity of guava puree during storage at 4°C. (○) pressurized at 600 MPa; (●) pressurized at 400 MPa; (Δ) heated at 88–90°C; (▲) without treatment (From Gow, C.Y., and Hsin, T.L. 1996. *Intl. J. Food Sci. Technol.* 31: 205–213. With permission)

### **2.1.9 Kiwi Fruit**

Actinidin is the sulfhydryl protease of the kiwi fruit and can be employed in place of other plant sulfhydryl proteases like papain and ficin as a milk clotting enzyme for traditional and novel dairy products, as meat tenderizer, and beer clarifier. Katsaros et al. (2009) demonstrated that high pressure resulted in controlled inactivation of actinidin and caused the desirable extent of clotting or tenderization. High pressure (200–800 MPa, 25–50 °C) induced inactivation allowed the selection of optimal high pressure process conditions for achieving desirable enzyme activity.

### **2.1.10 Lemons**

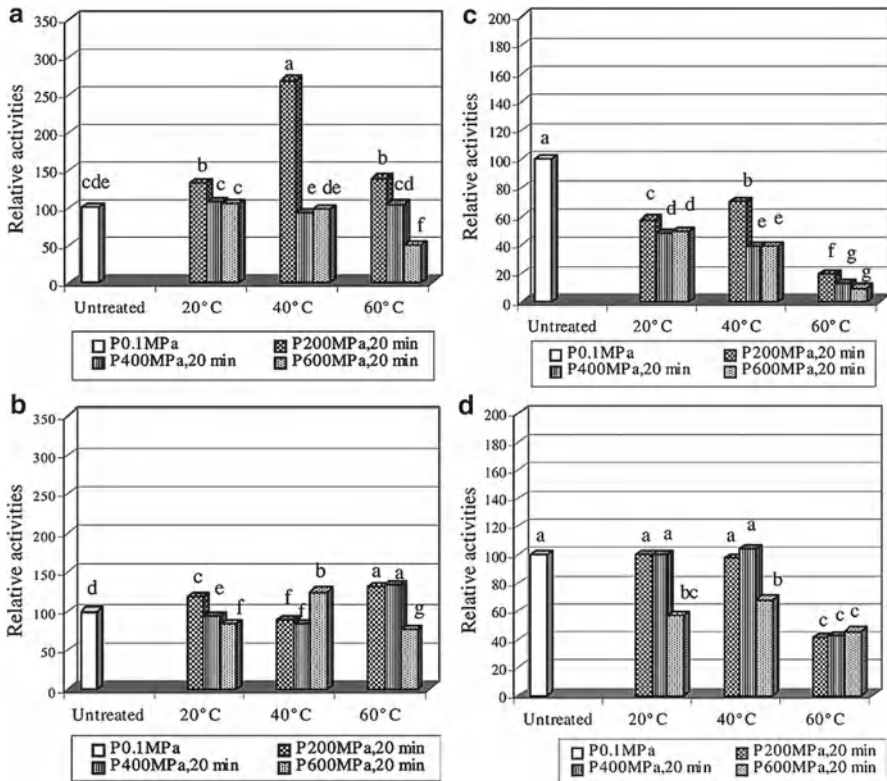
High-pressure-processed (300 MPa) lemon juice was demonstrated to have a satisfactory shelf life with minor changes in its constituents and physicochemical properties. No fungi were detected in pressure-treated lemon juices, whereas control samples were spoiled by yeasts and fungi after 10 days (Donsi et al. 1998). High-pressure (100 and 200 MPa) induced enzymatic treatment (combination of cellulase and xylanase) resulted in higher pectin yield from dried lime peel than those using acid and aqueous extraction only weakly affecting the average molecular weight and intrinsic viscosity of pectin extracts (Naghshineh et al. 2013).

### **2.1.11 Lychee**

The visual quality in both fresh and syrup-processed lychee fruit (*Litchi chinensis* Sonn.) after HPT (600 MPa at 60 °C for 20 min) was superior to that in the case of thermal processing. It led to extensive inactivation of POD and PPO in fresh lychees; these effects were less significant when the samples were processed in syrup (Fig. 2.8, Phunchaisri and Apichartsrangkoon 2005). Lychee fruit pericarp contains high amounts of flavonoids which are useful natural antioxidants. Prasad et al. (2009a, b) found that high-pressure extraction increases extraction yield (30 %) as compared to ultrasound-assisted extraction (24 %) and conventional extraction (1.83 %).

### **2.1.12 Longan**

Yang et al. (2009) indicated that high-pressure extraction of longan fruit (*Dimocarpus longan* Lour.) pericarp resulted in decrease in the yield of water-soluble polysaccharides (lowest being 6.4 mg/g at 500 MPa), whereas the yield of alkali-soluble polysaccharides and cellulose did not change significantly as compare to conventional extraction. The lignin composition also was not affected by the application of high

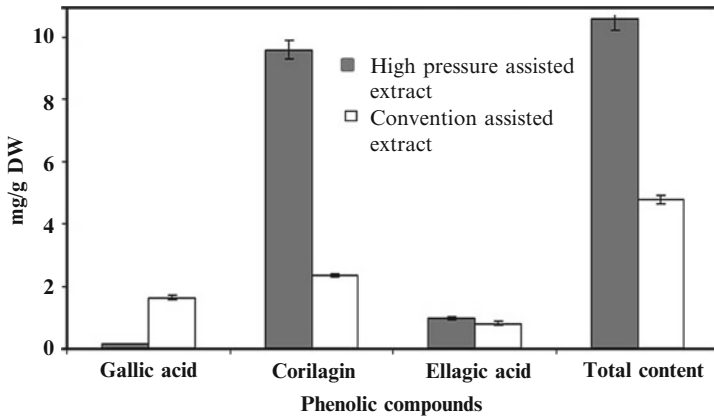


**Fig. 2.8** Effect of combined UHP/temperature on (a) POD and (b) PPO activity of fresh lychee; (c) POD and (d) PPO activity of syrup lychee (Phunchaisri, C., and Apichartsrangkoon, A. 2005. *Food Chem.* 93: 57–64. With permission)

pressure during extraction. HPT resulted in higher extraction of phenolic antioxidant compounds and less extraction time as compared to conventional extraction. Corilagin concentrations were highest among the three phenolic compounds namely gallic acid, corilagin, and ellagic acid. The total phenolic content of the high-pressure-assisted extract was also higher as compared to the conventional extract (Prasad et al. 2009c, d) (Fig. 2.9).

### 2.1.13 Mangoes

HPT (300 or 600 MPa, 1 min) of pre-cut mangoes during storage at 3 °C led to slightly reduced fresh flavor, increased off-flavor and sweetness, as well as improved microbial status, but color, texture, and other sensory attributes changed only slightly (Boynton et al. 2002). In the case of high-pressure shift freezing, before pressure release the entire volume of mango sample reached the initial freezing



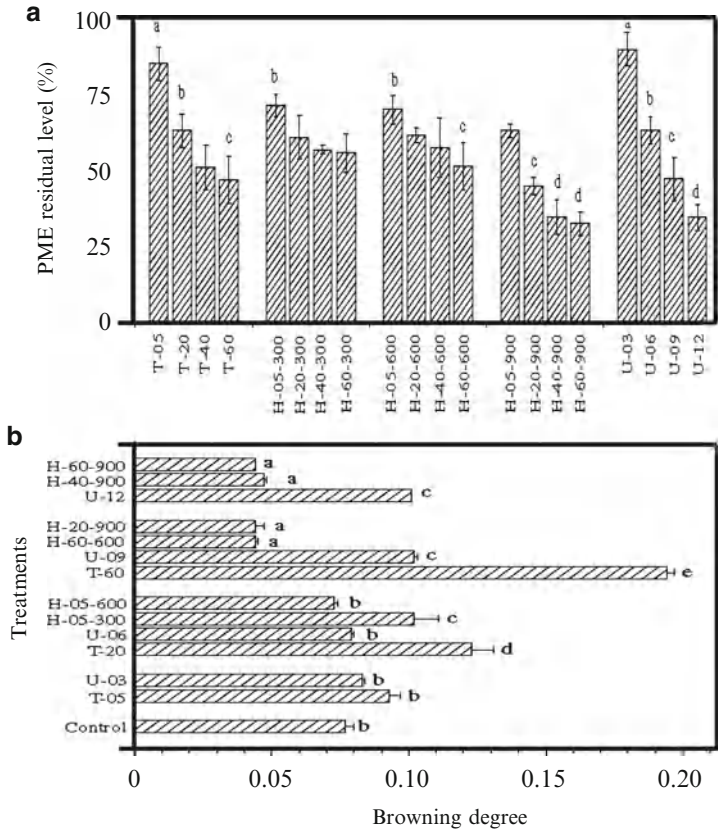
**Fig. 2.9** Phenolic contents of longan fruit obtained by different extraction methods (Prasad, K.N., Jing, H., Shi, J., Ting, L., Jiang, L., Xiaoyi, W., Shengxiang, Q., Xue, S., Yueming, J. 2009c. *Innovat. Food Sci. Emerg. Technol.* 10: 413–419. With permission)

point at the same time, which led to high levels of supercooling resulting in uniform and rapid ice nucleation throughout the sample volume, resulting in maintenance of original tissue microstructure (Otero et al. 2000).

HPT (552 MPa, 5 min) of mango puree with added ascorbic acid and phosphoric acid (pH 3.5) resulted in reduced rates of browning during storage at 3 °C for 1 month without any microbial growth (Guerrero-Beltran et al. 2006). Flow behavior index for fresh and canned mango pulp was found to decrease and increase, respectively, with increase in pressure treatment. At the same time, the consistency index of fresh pulp increased with pressure level from 100 to 200 MPa, while a steady decrease was observed for canned pulp (Ahmed et al. 2005). Aguirre et al. (2011) showed that mesophiles in fresh mango nectar were inactivated up to 4 log during come-up time of pressure application. The treatment at 345 and 414 MPa for 2 and 1 min, respectively, inactivated all viable *Escherichia coli*. The highest inactivation of mesophiles (7 log) was reported at 414 MPa after 4 min. No significant reductions in PME activity were observed after treatment at 275 or 345 MPa, but it was found to increase after treatment at 414 MPa. Hiremath and Ramaswamy (2012) pointed out that HPT at 400 MPa for 5 and 10 min for *Listeria mesenteroides* and *Escherichia coli* resulted in complete destruction and 6-log reduction, respectively. Complete eradication of *Escherichia coli* was achieved when the juice was subjected to 500 MPa for 1 min.

### 2.1.14 Melons

Wolbang et al. (2008) found that HPT of melon (*Cucumis melo* L.) did not have any effect on total titratable acidity and total soluble solids; however, color, ferric ion-reducing capacity, and vitamin C levels were adversely affected, while the



**Fig. 2.10** (a) Effect of treatments on the PME residual level. The *a*, *b*, *c*, and *d* represent (100±5)%, (75±5)%, (50±5)%, and (35±5)% of the PME residual level of the watermelon juice, respectively, after each treatment. (b) Effect of treatments on the browning degree of the watermelon juice. Data are means±standard deviation. Means with different letters represent a significant difference ( $P<0.05$ ) (Zhang, C., Trierweiler, B., Li, W., Butz, P., Xu, Y., Ruefer, C.E., Ma, Y., Zhao, X. 2011. *Food Chem.* 126: 254–260. With permission)

$\beta$ -carotene level was significantly increased. Zhang et al. (2011) demonstrated that HPT (600–900 MPa) was effective in inactivating the PME of watermelon juice, while browning degree and dynamic viscosity of the treated juice was comparable to the controls (Fig. 2.10). Furthermore, HPT had a slight impact on the all-*trans*-lycopene, total *cis*-lycopene, and total lycopene concentration of the juice.

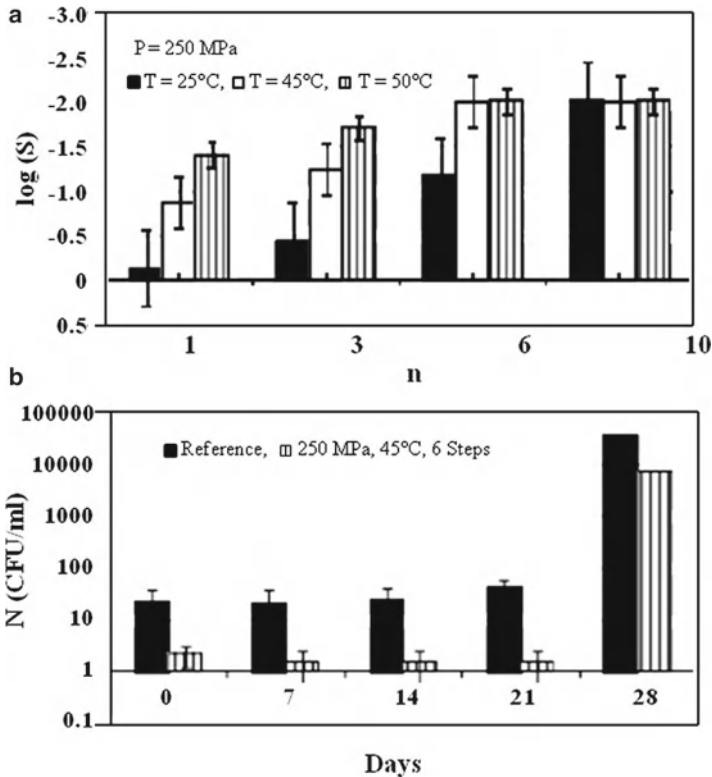
Cantaloupe (*Cucumis melo* L.) is a flavorful fruit with a unique aroma produced all around the world. However, because of lack of processing techniques, large amounts of cantaloupes rot away in farmlands every year. The fresh juice has a short shelf life and is highly heat sensitive. Ma et al. (2010) suggested that HPT may become a promising way to process cantaloupe juice. The microbial

count of juice after HPT (500 MPa, 20 min) was reduced to 100 CFU/100 ml and the activities of POD, PPO, and LOX were significantly lowered without change in sensory quality.

### 2.1.15 Oranges

Many researchers have reported extended shelf life of high-pressure-processed orange juice under refrigeration with increased flavor retention (Parish 1998a, b; Donsi et al. 1996; Strolham et al. 2000; Takahashi et al. 1998; Plaza et al. 2006a). HPT (800 MPa, 25 °C, 1 min) was shown to have a potential for stabilizing fresh orange juice yielding lowest levels of residual PME activity, good cloud stability, and less loss of ascorbic acid over a period of more than 2 months at 4 °C or 37 °C (Nienaber and Shellhammer, 2001). Later, Sampedro et al. (2008) demonstrated that HPT (700 MPa, 55 °C, 2 min) can result in complete inactivation of PME. High-pressure-processed (600 MPa, 1 min) juice from Valencia and navel oranges was shown to be safe for consumption and retained its freshness and nutritional values even after of 12 weeks at 4 °C (Sellahewa 2002). In case of Navel and Valencia orange juices the population of aerobic bacteria, yeasts, and other fungi was reduced to below detectable levels. Inactivation of *Salmonella* up to 7 log cycles and marked reduction of PME was also observed. Color, browning index, viscosity, °Brix and titratable acidity, levels of alcohol insoluble acids, ascorbic acid, and  $\beta$ -carotene were unaffected when stored for 12 weeks at 4 or 10 °C (Bull et al. 2004). Katsaros et al. (2010b) studied the inactivation kinetics of PME and pressure-resistant species of spoilage lactic acid bacteria in freshly squeezed Valencia orange juice under high pressure combined with moderate temperature. Process conditions of 350 MPa at 35 °C for 2 min were proposed for the cold pasteurization Valencia orange juice. Donsi et al. (2010) demonstrated the use of pulsed high hydrostatic pressure with moderate temperature (250 MPa, 45 °C, 6 pulses) to achieve a desired lethality. At this optimum condition, the natural freshness (color, odor aroma) as well as nutritional quality of orange juice was found to be preserved for 21 days at a storage temperature of 4 °C (Fig. 2.11).

No significant difference in antioxidative capacity, sugar and carotene contents between high-pressure and thermally pasteurized orange juice was shown (Fernandez et al. 2001a). HPT (500 MPa, 5 min, 35 °C) of orange juice resulted in lower loss of ascorbic acid as well as higher retention of flavor, antioxidant capacity, shelf life, sensory scores, and viscosity as compared to conventionally pasteurized samples (Polydera et al. 2003, 2004, 2005). The odor and flavor (volatile content, 20 key aroma compounds) of the high-pressure-processed orange juice was acceptable to consumers even after storage for 12 weeks at lower temperatures of up to 10 °C (Baxter et al. 2005). Pan et al. (2011) demonstrated that limonene degradation in case of freshly squeezed navel orange juice increased with increasing processing pressure or temperature. The limonene degradation was found to result in significant increase of  $\alpha$ -terpineol and carvone concentrations.

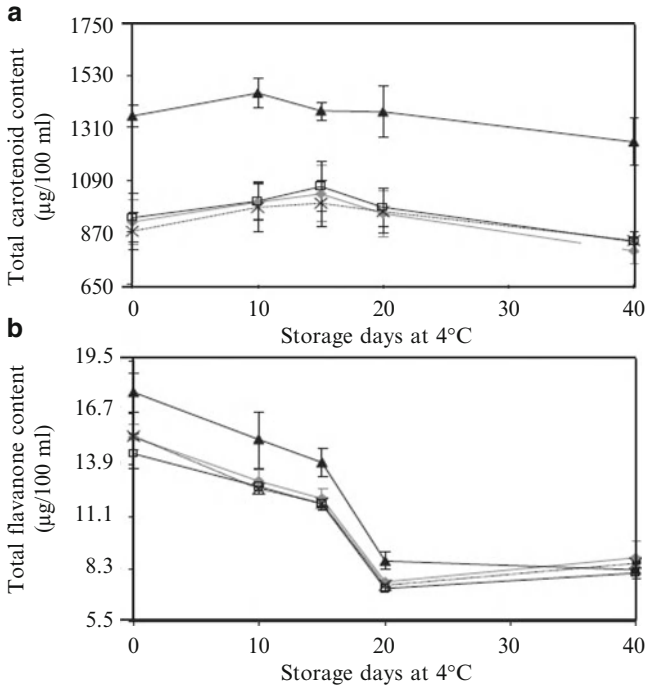


**Fig. 2.11** Levels of inactivation of freshly squeezed orange juices processed in pulsed HPT at (a) different temperatures and constant pressure, 250 MPa and (b) number of surviving cells during the shelf life of untreated (reference) and processed in pulsed HPT at different storage times at 4 °C. (Pulse holding time = 60 s, ramp rate = 10.5 MPa/s) (From Donsi, G., Ferrari, G., Maresca, P. 2010. *J. Food Sci.*75: E169–E177. With permission)

Ancos et al. (2002) showed that HPT (350 MPa, 5 min, 30 °C) leads to an increased extraction of carotenoids as compared to controls. Moreover, Sanchez et al. (2003), Sanchez et al. (2005) demonstrated increased extraction of health-promoting compounds such as flavanones, vitamin C, carotenoids, and antioxidants in orange juice during storage at 4 °C. Butz et al. (2004) demonstrated that excess ascorbate resulted in no major loss in folate (hematopoietic vitamin) in freshly squeezed orange juice during HPT (600 MPa, 80 °C).

Vervoort et al. (2011) compared the impact of high pressure with that of a pulsed electric field processing and thermal processing. None of the methods were able to cause a complete inactivation of PME, although heat and high pressure were the most effective in limiting the residual activity, whereas POD was completely inactivated by heat treatment and was much less susceptible to the other two methods. Timmermans et al. (2011) also compared HPT with mild heat pasteurization and pulsed electric field processing and indicated that thermal pasteurization resulted in





**Fig. 2.12** Profile of (a) total carotenoid content (b) total flavanone content in orange juices during storage at 4 °C for each treatment assayed. *FS* freshly squeezed (without treatment) (◆), *LPT* low pasteurization (70 °C/30 s) (×), *HP* high pressure (400 MPa/40 °C/1 min) (▲), *PEF* pulsed electric fields (35 kV cm<sup>-1</sup>/750 ms) (□) (From Plaza, L., Moreno, C.S., Ancos, B.D., Martínez, P.E., Belloso, O.M., Cano, M.P. 2011. *Lebens. Wiss. Technol.* 44: 834–839. With permission)

most stable orange juice in terms of cloud stability due to inactivation of PME. However, a lower cloud degradation rate was found for high-pressure-processed juice as compared to pulsed-electric-processed juice. Plaza et al. (2011) indicated that high-pressure pasteurization was more effective in preserving bioactive compounds in orange juice during refrigerated storage as compared to pulsed electric field as well as thermal pasteurization. Immediately after treatment, high-pressure-processed juice showed a significant increase in vitamin A, as well as total carotenoid and flavanone content, whereas no significant changes were observed for the other two treatments. Flavanone content was found to decrease significantly during the first 20 days of storage at 4 °C for all treatments, while carotenoid content showed a moderate decrease that took place during the last 20 days (Fig. 2.12).

Torres et al. (2011) pointed out that retention of anthocyanins and ascorbic acid of high-pressure-processed blood orange juice during processing was more than 99 % and 94.5 %, respectively. However, degradation of these compounds was found to follow first-order kinetics during storage. During storage at 4 °C for 10 days, the retention of anthocyanins and ascorbic acid of high-pressure-treated



(600 MPa, 15 min) orange juice was found to be 93.4 % and 85.0 %, respectively. Gou et al. (2012) demonstrated that high pressure resulted in increased yield of pectin (20.44 %) as compared to yields obtained by traditional (15.47 %) or microwave (18.13 %) heating. The intrinsic viscosity and average molecular weight of pectin extracted by HPT was much higher than those extracted by traditional heating or microwave compared to commercial pectin.

### **2.1.16 Passionfruit**

HPT (300 MPa, 5 min, 25 °C) was used to preserve yellow passion fruit pulp, which yielded a ready-to-drink juice with improved sensory quality free from cooked and artificial flavor attributes compared to commercial juices. HPT did not cause significant modifications in compounds responsible for the aroma, flavor, and consistency (Laboissiere et al. 2007).

### **2.1.17 Peaches**

HPT (>300 MPa) in combination with citric acid (1–1.2 %w/w) could effectively be used for inactivation of peach PPO enzyme, which indicating that such treatment could be a potential alternative for conventional blanching. Furthermore, the use of citric acid as carrier fluid resulted in an increased rate of removal of moisture resulting from the formation of cracks in the upper layer by the acidic medium (Kingsly et al. 2009b).

The inactivation of endogenous PME of Greek commercial peach pulp under high pressure (100–800 MPa, 30–70 °C) followed first-order kinetics. High pressure and temperature acted synergistically on PME inactivation, except at 70 °C within the middle pressure range (100–600 MPa), whereas antagonistic effects of pressure and temperature were observed (Boulekou et al. 2010). The development of brown color (measured as browning index) in peach puree subjected to HPTs (400–600 MPa, 1–3 min) during 6 weeks of storage at 4 °C and 20 °C followed a zero-order kinetic (Khalil et al. 2011).

### **2.1.18 Pears**

Beltran et al. (2011) demonstrated that HPT (0–241 MPa, 2 s, 0–15 min) reduced the initial counts in pear nectar inoculated with *Saccharomyces cerevisiae*, *Escherichia coli*, and *Listeria innocua* from  $6.0 \times 10^5$ ,  $1.02 \times 10^7$ , and  $2.4 \times 10^7$  CFU/ml to  $2.4 \times 10^5$ ,  $6.3 \times 10^5$ , and  $2.2 \times 10^7$  CFU/ml, respectively. The come-up time had an important microbial inactivation effect. The corresponding decimal reduction

time values were in the range of 2.0–35.3, 0.6–20.6, and 9.2–588.2 min, while  $Z_p$  values were 120.5, 92.6, and 75.2 MPa. Kou et al. (2012) showed that the volatile compounds in five pear cultivars differ considerably. The concentration of 1-methylcyclopropene could keep the levels of volatile compounds basically unchanged during storage and HPT changed the levels of volatiles significantly during the storage period.

### 2.1.19 Persimmons

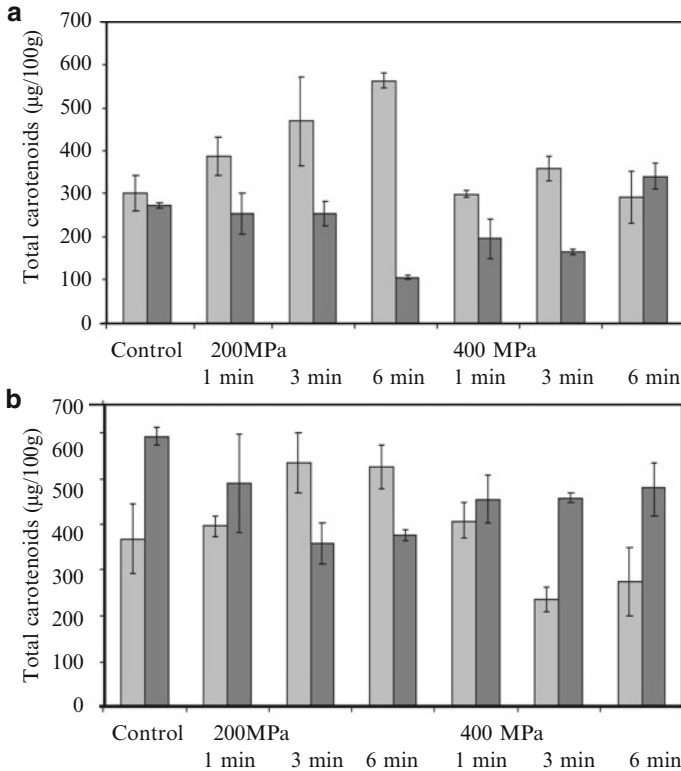
Persimmon fruit are an important source of phenolic compounds, dietary fiber, and carotenoids. HPT (up to 400 MPa) of persimmon puree resulted in increased amount of extractable carotenoids, which was related to the increase in vitamin A value, but, this did not corroborate with the increase in antioxidant activity (Ancos et al. 2000). Gutierrez et al. (2011) showed that application of high pressure resulted in cell wall disruption and intracellular component dispersion throughout the tissue, together with some nutritionally active compounds namely tannins, fiber, and carotenoids.

Rojo Brillante is an astringent variety of persimmon fruit that needs a de-astringency treatment (95 % CO<sub>2</sub>, 20 °C, 24 h) before commercialization to improve its sensorial quality. This fruit is a good source of bioactive compounds such as carotenoids. Plaza et al. (2012) studied the effect of HPT (200–400 MPa) on carotenoid content of astringent and nonastringent persimmon fruits at two maturity stages (III and V). HPT at 200 MPa resulted in an increase in extracted carotenoid content for astringent samples up to 86 % and 45 % at maturity stages III and V, respectively, whereas no significant differences or even a decrease was observed for nonastringent ones or those treated at 400 MPa (Fig. 2.13).

HPT (200–400 MPa, 1–6 min) of astringent and non-astringent persimmon ‘Rojo Brillante’ resulted in microstructural changes, which were related to the improvement in the diffusion and extractability of tannins and acid compounds. The application of high pressure resulted in decrease in flesh firmness and cohesiveness, while pH was increased in both astringent and non-astringent samples (Gutierrez et al. 2012).

### 2.1.20 Pineapple

Buzrul et al. (2008a) demonstrated that pineapple juice subjected to pulsed HPT (300 MPa, 20 °C, 60 s, 5 pulses) resulted in significant inactivation of *Escherichia coli* and *Listeria innocua* at lower pressure values than the ones used in commercial applications (>400 MPa). However, the pressure-treated juice stored at 4 °C, 20 °C and 37 °C up to 3 weeks led to an increase in the level of microbial inactivation and no injury recovery of the bacteria were detected. Ascospores of *Byssoschlamys nivea* are extremely heat-resistant and frequently associated with the deterioration of

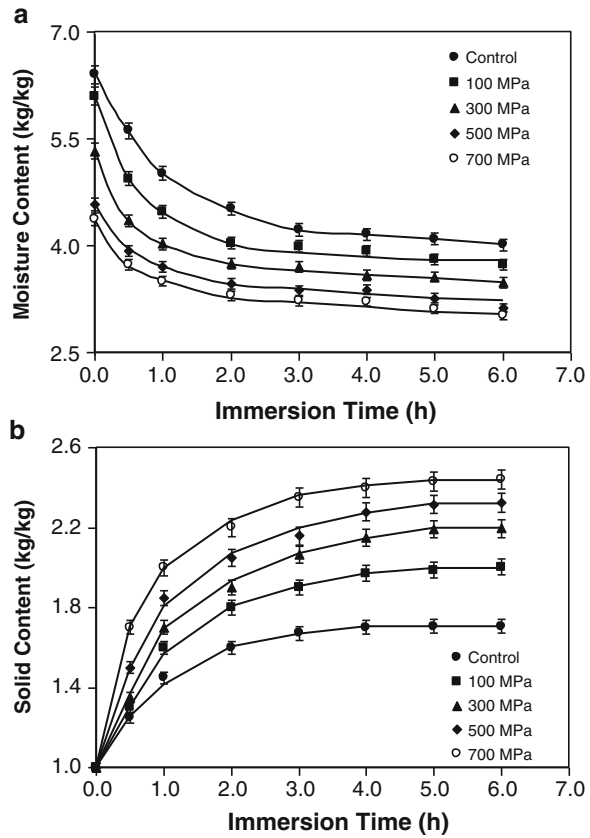


**Fig. 2.13** Total carotenoid content of HP-treated (at 25 °C) astrigent and nonastrigent persimmon fruits. (a) Maturity stage III and (b) maturity stage V. (■) Astrigent, (■) nonastrigent (From Plaza, L., Colina, C., Ancos, B. de, Sanchez, M.C., Cano, M.P. 2012. *Food Chem.* 130: 591–597. With permission)

thermally treated fruit products. Ferreira et al. (2009) demonstrated that pressure cycles were more effective for inactivating *Byssochlamys nivea* ascospores in pineapple juice and nectar than the application of sustained high pressures. The ascospores were inactivated by applying sustained pressure of 600 MPa at 90 °C for 15 min and pressure cycles at 600 MPa and 80 °C for three cycles of 5 min or five cycles of 3 min could inactivate  $10^5$ – $10^6$  CFU/ml of the ascospores in pineapple juice and nectar.

Application of HPT (100–800 MPa) was reported to enhance water removal as well as solute gain during osmotic dehydration of pineapple. Water as well as solute diffusivity values were reported to be increased by a factor of four and two, respectively. The compression and decompression steps during pressurization and release of pressure, respectively, caused the removal of a significant amount of water, which was attributed to cell wall rupture (Rastogi and Niranjana 1998, Fig. 2.14). Kingsly et al. (2009a) reported that application of high pressure reduced sample hardness, springiness, and chewiness, while it had no significant effect on cohesiveness of

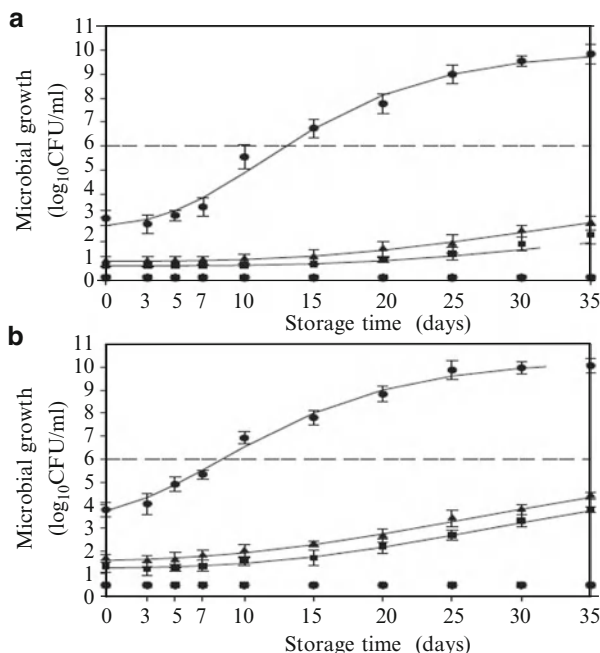
**Fig. 2.14** (a) Variation of moisture and (b) solid content (based on initial dry matter content) with time during osmotic dehydration (From Rastogi, N.K., and Niranjana, K. 1998. *J. Food Sci.* 63: 508–511. With permission)



pineapple. Moreover, the treatment reduced the drying time of pineapple slices. The effective moisture diffusivity was found to increase with an increase of pressure up to 500 MPa. Rastogi et al. (2000a) demonstrated that high-pressure-pretreated pineapple subjected to osmotic dehydration and then dehydration resulted in a dried product having less solid diffusion during rehydration, and so was the release of the cellular components. The reduction in loss of soluble solids during rehydration was due to formation of a gel-network between divalent ions and de-esterified pectin (Basak and Ramaswamy 1998; Eshtiaghi et al. 1994). It may prove to be a useful technique to reduce the loss of nutrients or color from dehydrated product during rehydration.

### 2.1.21 Pomegranate

Ferrari et al. (2010) demonstrated that HPT (400–600 MPa) of pomegranate juice at room temperature increased the intensity of red color of the fresh juice and preserved the natural anthocyanins content. The operating pressure, temperature, and



**Fig. 2.15** Growth curve of (a) aerobic mesophilic and (b) molds and yeasts in untreated and high-pressure-treated pomegranate juice during storage at 4 °C for 35 days. Control (●), 350 MPa/30 s (▲), 350 MPa/90 s (■), 350 MPa/150 s (▼). The dotted line shows upper acceptable limit (Santos, E.V., Martinez, A.O., Munizaga, G.T., Reyes, J.E., Won, M.P., Labarca, V.B., Castro, J.M. 2012. *Innovat. Food Sci. Emerg. Technol.* 13: 13–22. With permission)

holding times were optimized with the aim of optimizing the processing condition in order to assure the microbiological stability, minimum degradation of the anthocyanins, as well as inactivation of PPO activity. Santos et al. (2012) indicated that pomegranate juice after HPT (>350 MPa, 150 s) resulted in a reduction of the microbial load around 4.0 log cycles, and the microbial populations (aerobic mesophilic bacteria as well as molds and yeasts) were below the detection limit during the entire storage period at 4 °C for more than 35 days (Fig. 2.15). The high-pressure-treated samples showed a slight reduction in antioxidant capacity during storage, while phenolic content increased. Total color difference ( $\Delta E$  values) showed significant differences in color between untreated and treated samples. The pH, total soluble solids, and titratable acidity of high-pressure-treated samples did not significantly change during the first 15 days of storage.

Romero et al. (2012) demonstrated that HPT (350–550 MPa) of pomegranate arils was able to reduce the initial microbial load to less than 1.0 CFU/g and shelf life was extended for more than 35 days. During storage time, the total polyphenol content and antioxidant activity were found to decrease significantly compared to controls.

## 2.2 Vegetables

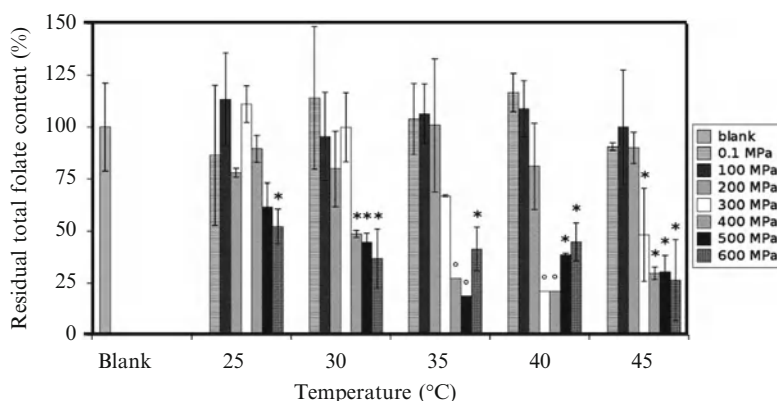
### 2.2.1 Bitter Melon

High-pressure extraction of momordicosides from fresh bitter melon (*Momordica charantia* L.) was found to be more efficient and rapid as compared to heat reflux extraction. The optimized parameters namely extraction pressure (423 MPa), extraction time (7 min), solvent to sample ratio (45.3:1 ml/g), and ethanol concentration of 70 % (vol./vol.) resulted in a maximum yield of 3.27 g Rg1 equivalents/100 g bitter melon dry weight (Ji et al. 2010).

### 2.2.2 Broccoli

Glucosinolates present in broccoli can be hydrolyzed by endogenous myrosinase to isothiocyanates, the latter exerting anticarcinogenic activity. High pressure (100–500 MPa, 40 °C) was shown to have significant effect on glucosinolate degradation (Eylen et al. 2007, 2009; Barba et al. 2010). Verlinde et al. (2008) indicated that folylpoly- $\gamma$ -glutamates present in broccoli were converted to folylmono- and folyldi- $\gamma$ -glutamates by HPT (up to 600 MPa, 25–45 °C), which impairs dietary folate bioavailability (Fig. 2.16). HPT (500 MPa, 10 min) preserved nutritional substances in apple-broccoli juices such as sulforaphane and antimutagenic activity, apart from microbial inactivation (Houska et al. 2006).

Butz et al. (2002) showed that HPT did not have a significant impact on chlorophyll a and b in broccoli. The flavor of pressure-shift-frozen broccoli samples was



**Fig. 2.16** Influence of thermal (25–45 °C, 0.1 MPa, 30 min) and isothermal-isobaric treatments (25–45 °C, 100–600 MPa, 25 min) on total folylpoly- $\gamma$ -glutamate content in broccoli. \*Indicates significant difference with blanks ( $P < 0.05$ ) (From Verlinde, P., Oey, I., Hendrickx, M. and Loey, A. van. 2008. *Food Chem.* 111: 220–229. With permission)

not acceptable after 30 days of storage at  $-20\text{ }^{\circ}\text{C}$ , even though the texture remained quite firm. However, the sensory quality of samples blanched prior to high-pressure freezing (210 MPa,  $-20\text{ }^{\circ}\text{C}$ ) was acceptable (Prestamo et al. 2004). Blanched and high-pressure-frozen broccoli presented less cell damage, lower drip losses, and better texture than frozen samples (Fernandez et al. 2006).

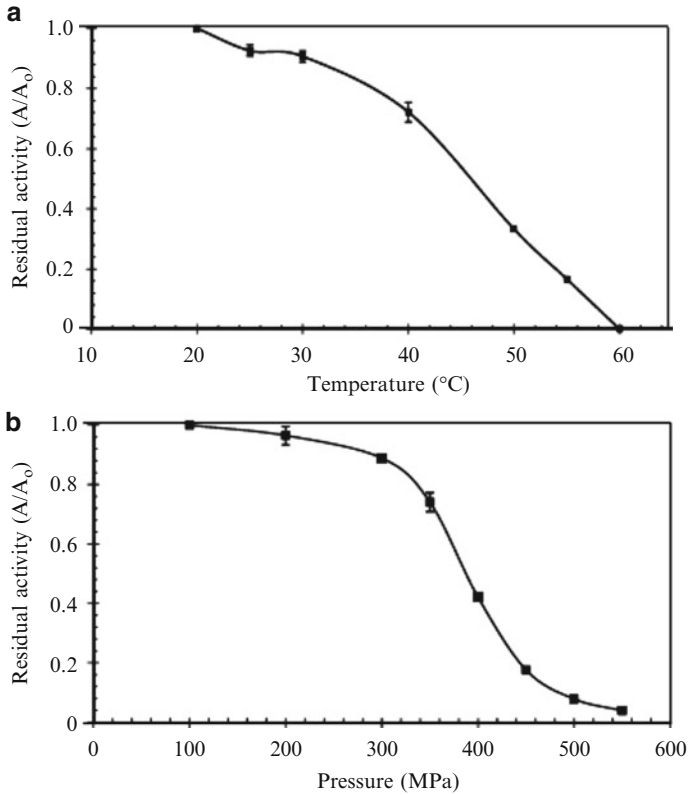
### 2.2.3 Cabbage

The application of high pressure (up to 500 MPa) to white cabbage resulted in reduction in the proportion of soluble fiber; total fiber content remained constant, which resulted in producing white cabbage with specific properties in terms of nutrition and function (Wennberg and Nyman 2004). HPT (600 MPa) was shown as an alternative preservation method for sour Chinese cabbage. The pressure level of 200 MPa had no significant impact on total aerobic bacteria, lactic acid bacteria, and yeasts in sour Chinese cabbage. The surviving total aerobic bacteria and lactic acid bacteria at 400 MPa equaled initial counts after 15-day storage at  $27\text{ }^{\circ}\text{C}$  and  $37\text{ }^{\circ}\text{C}$ , whereas they were inhibited at  $4\text{ }^{\circ}\text{C}$  up to 60 days. The surviving total aerobic bacteria at 600 MPa did not grow. Yeasts at 400 and 600 MPa decreased below detectable levels after 2 days during storage (Li et al. 2010).

During storage of sauerkraut (obtained from white cabbage by natural or induced fermentation), a gradual rise of aerobic mesophilic bacteria and lactic acid bacteria was observed. HPT led to a reduction in microbial counts ( $4\text{--}5\text{ log CFU/g}$ ), which were further increased during storage. But the counts were always less than that of unpressurized sample stored for the same period (Penas et al. 2010). Koo et al. (2011) demonstrated that glucoraphanin present in red cabbage can be hydrolyzed by myrosinase to form sulforaphane, which has a cancer chemopreventive activity. The HPT at 400 MPa, followed by incubation at  $60\text{ }^{\circ}\text{C}$  resulted in highest concentration of sulforaphane ( $99.7\text{ }\mu\text{mol/kg}$  fresh wt). Ghawi et al. (2012) showed that the combined high-pressure (100–400 MPa) and temperature ( $35\text{--}50\text{ }^{\circ}\text{C}$ ) treatment followed first-order myrosinase inactivation kinetics. The results indicated that green cabbage myrosinase was stable up to  $35\text{ }^{\circ}\text{C}$  and decay in activity occurred at higher temperatures, whereas it was stable up to 250 MPa and inactivation commenced from 300 MPa and above (Fig. 2.17).

### 2.2.4 Carrots

High pressure resulted in softening of carrot due to destruction of cell membrane and loss of soluble pectin along the cell liquor. PME resulted in de-esterification of pectin during depressurization and even after release of the pressure resulted in tissue hardening. High-pressure-processed carrot retained textural characteristics as compared to controls (Stute et al. 1996).

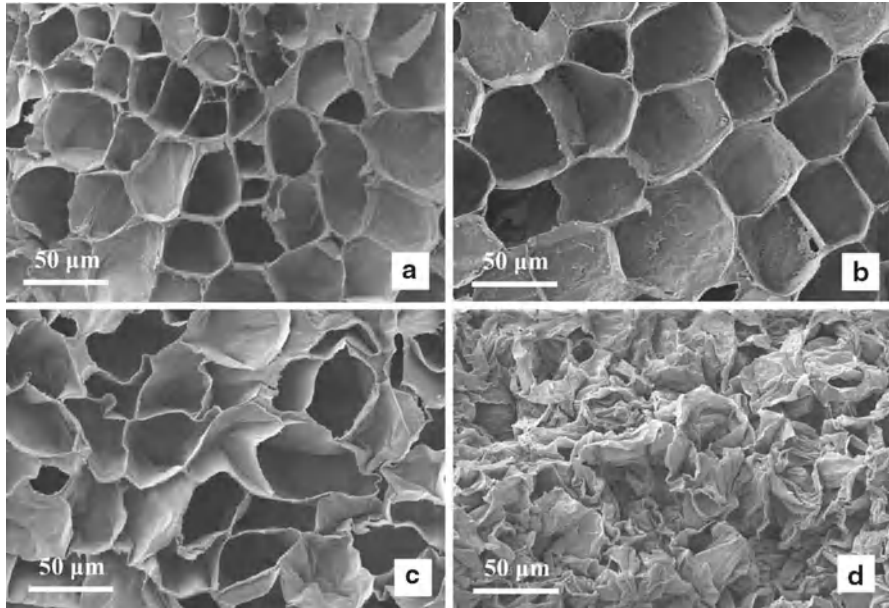


**Fig. 2.17** Effect of (a) temperature and (b) pressure on residual activity of myrosinase ( $A/A_0$ ) from green cabbage (From Ghawi, S.K., Methven, L., Rastall, R.A., Niranjani, K. 2012. *Food Chem.* 131: 1240–1247. With permission)

The instantaneous application of high pressure resulted in initial loss of texture followed by texture recovery as a result of pressure-hold. The extent of the initial loss of texture was more prominent at higher pressures and partial recovery of texture was higher at lower pressures. The texture reached original values at low pressure for long processing times (Basak and Ramaswamy 1998). The rapid loss of firmness of carrots was due to disruption of membranes, which reduces cell turgor pressure resulting in more deformable material or rubbery-like texture (Araya et al. 2007; Michel and Autio 2001). A combination of HPT with  $\text{CaCl}_2$  infusion resulted in texture improvement during thermal processing. High-pressure pretreatment alone resulted in less loss of texture when the sample was treated at 100–125 °C (Sila et al. 2004, 2005).

Pressure-assisted thermal processing (PATP, 500–700 MPa, 95–105 °C) resulted in less quality degradation (texture, color, and carotene content) as compared to thermally processed carrots (Nguyen et al. 2007) due to non-occurrence of





**Fig. 2.18** Microstructures of (a) control, (b) pressure-treated (700 MPa, 25 °C, 5 min), (c) pressure-assisted thermal processed (700 MPa, 105 °C, 5 min), and (d) thermal-processed (105 °C, 0.1 MPa, 30 min) carrot samples (From Nguyen, L.T., Rastogi, N.K., and Balasubramaniam, V.M., *Journal of Food Science*, 72, E269, 2007. With permission)

$\beta$ -elimination reaction and stimulation of demethoxylation of pectin (Roeck et al. 2008, 2009). Microstructures of cross-sections of raw, PATP (700 MPa, 105 °C, 5 min), and thermal processed (105 °C, 0.1 MPa, 30 min) carrot samples showed that the extent of structural damage was limited in the case of PATP, whereas thermal processing transformed intact cell structures of raw carrot to separated and ruptured cells with nondistinct middle lamellas because of degradation of pectinaceous material (Nguyen et al. 2007; Rastogi 2009b, Fig. 2.18).

Further, Roeck et al. (2009) demonstrated that combination of high pressure with high temperature (500–700 MPa, 90–115 °C) resulted in retardation or even stoppage of  $\beta$ -elimination reaction, whereas demethoxylation reaction was stimulated, this enhanced tissue strength by forming cross-links with divalent ions. Furthermore, hardness during pressure-assisted thermal processing could be further improved by a combined pretreatment involving calcium infusion (Rastogi et al. 2008a, b). The texture degradation in case of pressure-assisted thermally processed (600 MPa, 95–110 °C) sample was found to be tenfold slower in comparison to thermal treatment (Roeck et al. 2010).

The retention of total antioxidant activity, levels of ascorbic acid and carotenoids in carrot puree subjected to HPT (400–600 MPa, 15 min, 20 °C) were quite high as compared to thermal treatment (70 °C, 2 min). However, the color

parameters were significantly affected, whereas no significant change in phenolic content was observed by both of these processing techniques (Patras et al. 2009b). HPT (500–600 MPa) of carrot juice reduced the total counts by ~4 log cycles and only slight growth of the survivors was observed during storage at 4 °C up to 22 days. The total counts increased during storage of the product at 8 °C and 12 °C but it took significantly longer to reach maximum levels as compared to the untreated juice (Patterson et al. 2012).

The carrot sample did not freeze when subjected to high pressure (200–400 MPa) under freezing conditions (–30 °C); when pressure was reduced to atmospheric pressure, quick freezing was observed. These samples had better firmness, texture, and histological structure of frozen carrots than the ordinary frozen samples (Fuchigami et al. 1997a, b).

HP-processed (600 MPa, 2 min) carrots were found to be similar to sous-vide (90 °C, 5 min) carrots in terms of sweetness, green flavor, and crunchy texture. Furthermore, high-pressure carrots showed higher intensity perception of orange color and fibrousness and were shown to be better preserved for 14 days at 4 °C (Araya et al. 2009). A comparison of HPT with sous-vide-processed carrot disks indicated that HP-processed samples have higher retention of polyacetylenes. Falcarindiol-3-acetate and falcarindiol were found to be the most barosensitive and thermosensitive, respectively (Rawson et al. 2012).

### **2.2.5 *Cauliflower***

A considerable loss of turgor and structural collapse has been observed during HPT (400 MPa, 30 min, 5 °C) of cauliflower. The high pressure changes cell permeability and enables the movement of water from inside to outside the cell. As a result, treated tissue had a soaked or drenched appearance, however, after these changes, cauliflower maintained near-original, acceptable firmness and flavor (Prestamo and Arroyo 1998).

### **2.2.6 *Chestnut Kernels***

HPT at 300 and 500 MPa resulted in 61.3 % and 40.9 % retention of volatile compounds, respectively, in an instant chestnut product. The flavor profile of pressure-treated product indicated the appearance of new compounds and the disappearance of other compounds; but the characteristic flavor compounds were found to have high retention. The levels of aldehydes, ketones, and benzene compounds were decreased, but those of heterocyclic compounds and esters increased after pressure treatment (Zhiqing et al. 2011).

### **2.2.7 Eggplant**

High-pressure-frozen eggplant samples had the highest firmness and the lowest rupture strain and drip loss compared to those of air-frozen samples. It was attributed to the formation of heavy ice polymorphs resulting from freezing of water under high pressure (100–700 MPa) leading to volume reduction (Otero et al. 1998). Microgram of still-air-frozen and air-blast-frozen eggplant with fresh sample indicated cell separation and disrupted cell wall, whereas, HP-assisted frozen sample had appearance similar to fresh sample and all the cells were positioned together and no cellular damage was evident (Fig. 2.19).

### **2.2.8 Garlic**

HPT (600 MPa, 1 min) combined with citric or ascorbic acid (5 or 10 g/kg) treatment resulted in retarded browning of chopped garlic taken from dormant bulbs and stored under ambient conditions for 6 months. Also, the browning of germinated bulbs was reduced without inhibiting greening, which is a physiological disorder (Seok and Dong 2001). Ma et al. (2011) demonstrated the possibility of adjusting formation and degradation of the volatile compounds in garlic by pressurization to produce garlic products with different flavor. HPT at 200, 400, and 600 MPa for 20 min of intact garlic resulted in 29, 19, and 12 kinds of volatile compounds, respectively, where alkyl sulfides accounted for 61.04 %, 41.38 %, and 22.73 % in relative peak area. The flavor intensity of odor-active compounds decreased with an increase in pressure. The alliinase activity increased by 2.89 % after treatment of 200 MPa, but decreased by 37.19 % and 59.18 % after treatment of 400 and 600 MPa, respectively, leading to different flavor intensities of garlic.

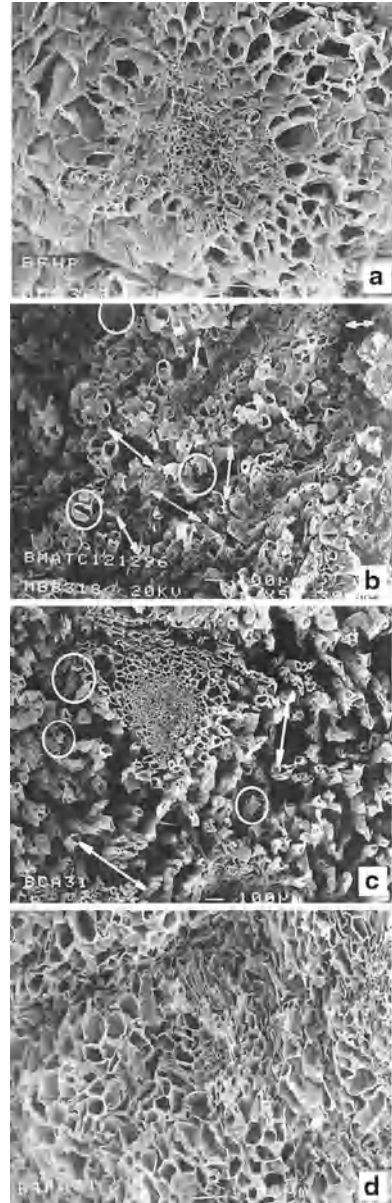
### **2.2.9 Ginger**

Yamaguchi et al. (2010) pointed out that HPT (400 MPa, 5 min) of grated ginger resulted in the inactivation of quality-degrading enzymes such as geraniol dehydrogenase (less than 5 %) and PPO (37 %). On the other hand, heat treatment (100 °C, 10 min) reduced geraniol dehydrogenase and PPO activity to 43 % and 10 %, respectively. In case of HP-treated ginger, terpene and aldehydes disappeared without the formation of alcohols. Browning was not observed immediately after HPT, whereas it was complete in heat-treated samples.

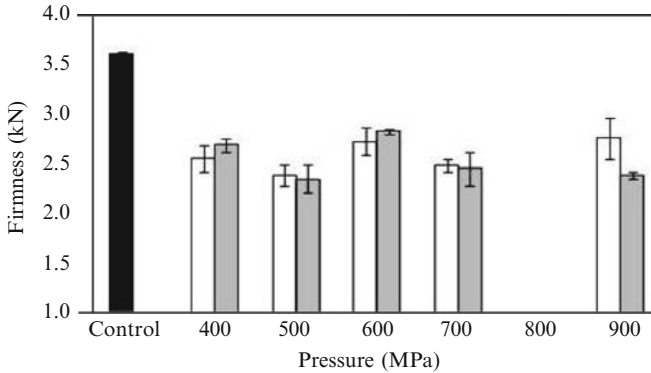
### **2.2.10 Green Beans**

HPT (500 MPa) and pulsed HPT (500 MPa, 2 pulses, 70 °C) resulted in improved texture, nutritional quality, and appearance of green beans and shelf life of beans

**Fig. 2.19** Scanning electron micrographs of eggplant (a) fresh; (b) still-air frozen tissue (c) air-blast frozen tissue (d) HP-frozen tissue. Cell separation and disrupted cell walls are marked by arrows and circles, respectively (From Otero, L., Solas, M.T., Sanz, P.D., Elvira, C. de and Carrasco, J.A. *Euro. Food Res. Technol.* 206: 340–341, 1998. With permission)



was extended to at least 1 month at 6 °C or 20 °C storage temperature Krebbers et al. (2002). A comparison of HP sterilization of beans with the equivalent thermal processing showed that HP-sterilized green beans were darker and greener in appearance, and also twice as firm as the thermally processed samples (Leadley et al. 2008).



**Fig. 2.20** Firmness of fresh and pressure-treated (5 or 10 min) green peas stored at  $-18^{\circ}\text{C}$  for a week and thawed at room temperature (From Quaglia, G.B., Gravina, R., Paperi, R., and Paoletti, F. 1996. *Lebens. Wissen. Technol.* 29: 552. With permission)

### 2.2.11 Green Peas

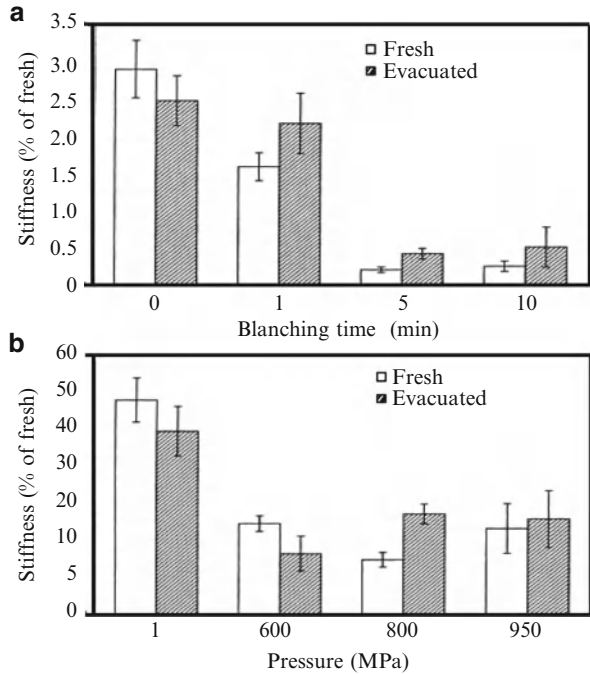
HPT (900 MPa) was demonstrated to be an alternative to thermal blanching of green peas. It resulted in a higher retention of ascorbic acid (82 %) in comparison with water (10 %) or microwave blanching (47 %). Although a significant softening resulted with respect to fresh frozen peas (Fig. 2.20). But, a combination of high pressure (400–900 MPa) with heat treatment (up to  $60^{\circ}\text{C}$ ) did not cause any significant changes in firmness (Quaglia et al. 1996).

### 2.2.12 Mushrooms

HPT (600–900 MPa) resulted in less texture degradation of mushrooms compared to thermal blanching (Fig. 2.21). High pressure resulted in permeabilization of cell membranes due to crystallization of phospholipids within the cell membranes. The increased permeability resulted in contact of extracellularly located PPO with phenols leading to enhanced browning. However, product yield and color were comparable to thermally blanched products. The intensity of browning was further reduced by evacuating mushrooms before pressurization (Matser et al. 2000).

HPT at 1,400 and 1,600 MPa for 1 min reduced PPO activity by 90.4 % and 99.2 % in phosphate buffer; however, higher enzyme activity remained in the mushroom puree after the treatment. Circular dichroism and fluorescence spectra analysis showed that the secondary and tertiary structures of HP-treated PPO were changed (Fig. 2.22). Sulfhydryl group content on the surface of HP-treated mushroom PPO was found to increase, which indicated that the inactivation of mushroom PPO must have resulted from the synergistic effect of the pressure and the heat arising from pressurization (Yi et al. 2012).

**Fig. 2.21** Stiffness of evacuated and fresh mushrooms after (a) blanching, (b) pressure treatment expressed as percentage of the stiffness of fresh mushrooms (From Matser, A.M., Knott, E.R., Teunissen, P.G.M., Bartels, P.V. 2000. *J. Food Eng.* 45: 11–16. With permission)



### 2.2.13 Olives

“Cornezuelo”-dressed olives are highly valued for their excellent organoleptic characteristics but have a low stability. HPT (400–600 MPa, 5 and 10 min) enhanced the shelf life of the olives prepared without preservatives. HP-treated olives showed higher stability and firmness but no significant differences were observed for color. The sample treated at 400 MPa for 5 min obtained higher score for sensory analysis after 120 days of storage (Pradas et al. 2012).

### 2.2.14 Onions

HPT (100 MPa) affected onion epidermis cells and cellular components, such as vacuoles. PPO oxidized phenol to orthoquinones, which upon polymerization formed brown pigment in diced onions. The rate of browning reaction was found to increase with an increase in pressure. Microscopic studies revealed that ability of sample to respond to sucrose was only affected to a minor degree for the samples treated at 100 MPa at 25 °C; however, severe damage to vacuoles of onion epidermis cells and cellular components was found for samples treated at 300 MPa (Butz et al. 1994). HPT (100 and 400 MPa, 5 °C) of onion led to better extraction of

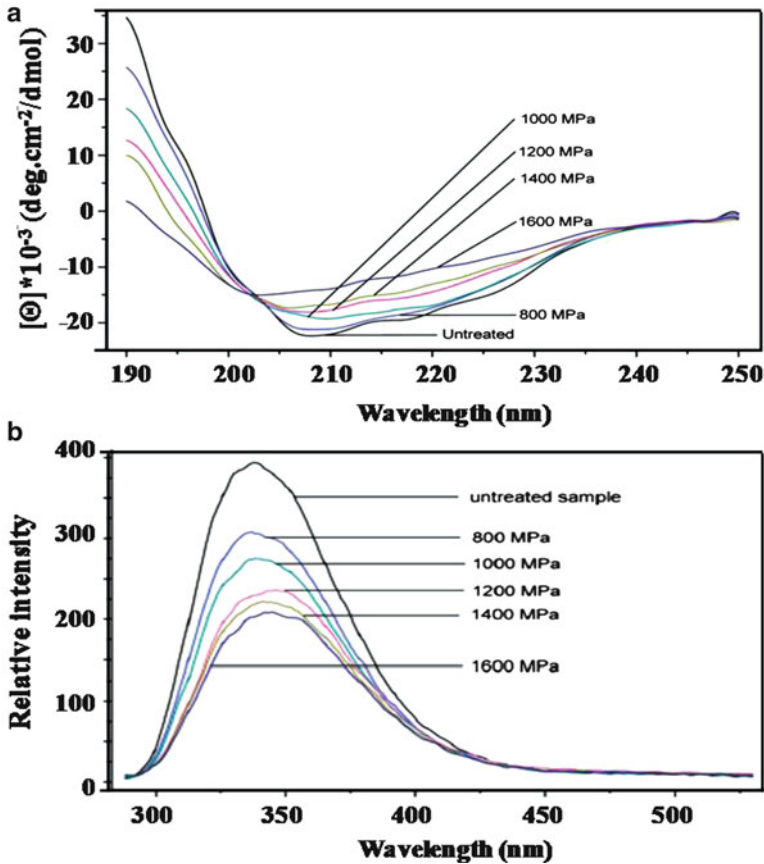


Fig. 2.22 (a) Far-UV CD spectra, (b) fluorescence emission spectra of high-pressure-treated mushroom PPO (From Yi, J., Bin J., Zhong, Z., Xiaojun, L., Yan, Z., Xiaosong, H. 2012. *J. Agric. Food Chem.* 60: 593–599. With permission)

flavonols (quercetin and quercetin glucosides) and increased antioxidant activity. At 400 MPa, the extraction of quercetin glucoside increased to 33 %, but there was no change in antioxidant activity (Roldan et al. 2009).

Gonzalez et al. (2010a) used proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) relaxometry to study the effects of HP and thermal processing on membrane permeability and cell compartmentalization. Loss of membrane integrity was clearly shown by changes in transverse relaxation time in thermally processed sample. Gonzalez et al. (2010b) also determined changes in cell membrane permeability and/or integrity by measuring formation of pyruvate as a result of membrane permeabilization as well as leakage of electrolytes into solution.

Neetoo et al. (2011) indicated that HPT (250–500 MPa, 2 min) of green onions in unwetted, wetted (briefly dipped in water), or soaked (immersed in water for 30 min) conditions reduced the population of *Salmonella* and *Escherichia coli*



O157:H7 by 0.6 to more than 5 log CFU/g. The extent of pressure inactivation increased in the order of soaked>wetted>unwetted state. Furthermore, Neetoo et al. (2012) showed that green onions grown in soil contaminated with *Escherichia coli* O157:H7 and *Salmonella* take up the pathogens in their roots, bulbs, stems, and leaves, but that HPT (400–500 MPa, 2 min) eliminated both of these pathogens.

### **2.2.15 Bell Peppers and Red Pepper**

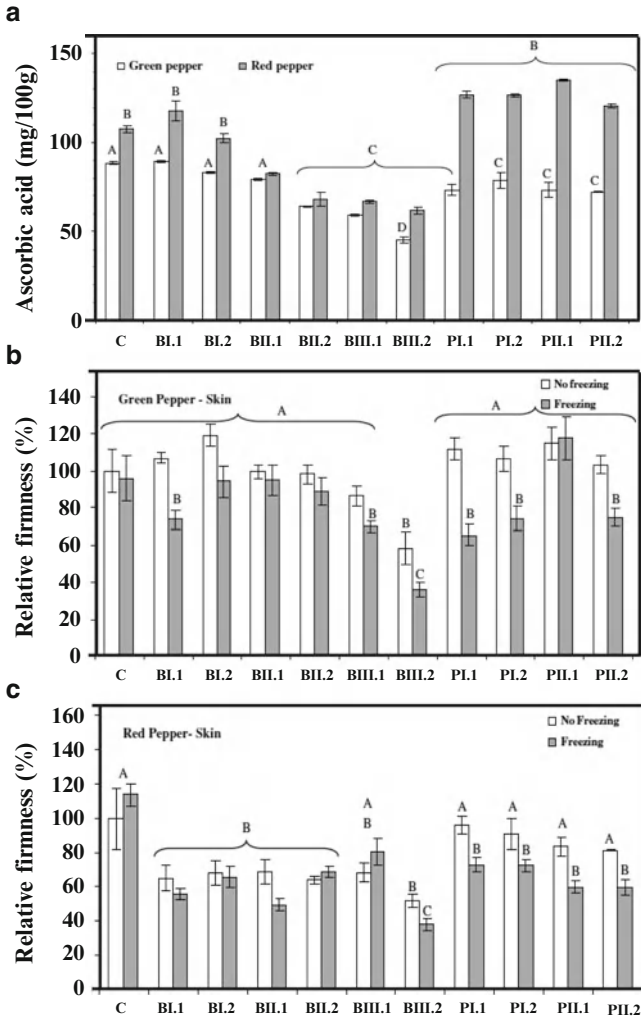
HP-pretreated *Capsicum annuum* (400 MPa, 10 min) resulted in higher drying rates for red pepper during dehydration. The pretreatment can be used as an alternative to chemical (NaOH or HCl) pretreatments thereby minimizing environmental pollution from chemicals (Ade-Omowaye et al. 2001). The use of HPT (100–200 MPa, 10–20 min) as an alternative pretreatment in place of blanching resulted in producing frozen bell peppers with better nutritional (ascorbic acid) and texture (firmness) characteristics (Fig. 2.23, Castro et al. 2008).

### **2.2.16 Potatoes and Sweet Potatoes**

High pressure (400 MPa, 15 min) in combination with citric acid (0.5 wt%) was shown as an alternative to hot water blanching. The treatment resulted in complete inactivation of PPO and reduction in microbial count by 4 log cycles (Eshtiaghi and Knorr 1993). HP-blanching samples resulted in a significant increase in drying rates due to cell permeabilization by HPT (Eshtiaghi et al. 1994). HPT enhanced the rate of dehydration of potato during osmotic dehydration (Rastogi et al. 2000b,c). The variation in cell disintegration index ( $Z_p$ ) of pressure-treated (200 and 400 MPa) and untreated potato samples with distance from the center of the material for different dehydration times indicated that the dehydration front moved faster within the pressure-treated sample (Fig. 2.24, Rastogi et al. 2003). Similarly, Sopanangkul et al. (2002) demonstrated the acceleration of osmotic dehydration of potato due to application of HPT up to 400 MPa. Further increase in pressure resulted in starch gelatinization leading to hindered diffusion. Yucel et al. (2010) indicated that high-pressure pretreatment (100–300 MPa) of carrots, green beans, and apple also resulted in higher drying rates during dehydration.

Abe et al. (2011) studied the effect of pretreatments such as heat treatment (100 °C, 5 min), heat treatment after HPT (200 MPa, 5 min), and HPT after heat treatment prior to drying of sweet potato on the rate of rehydration and quality. No significant difference in the color parameters or gelatinization rates of rehydrated sweet potato exposed to the three different pretreatments was observed. Heat treatment after HPT prior to drying was shown as an effective method for texture improvement.

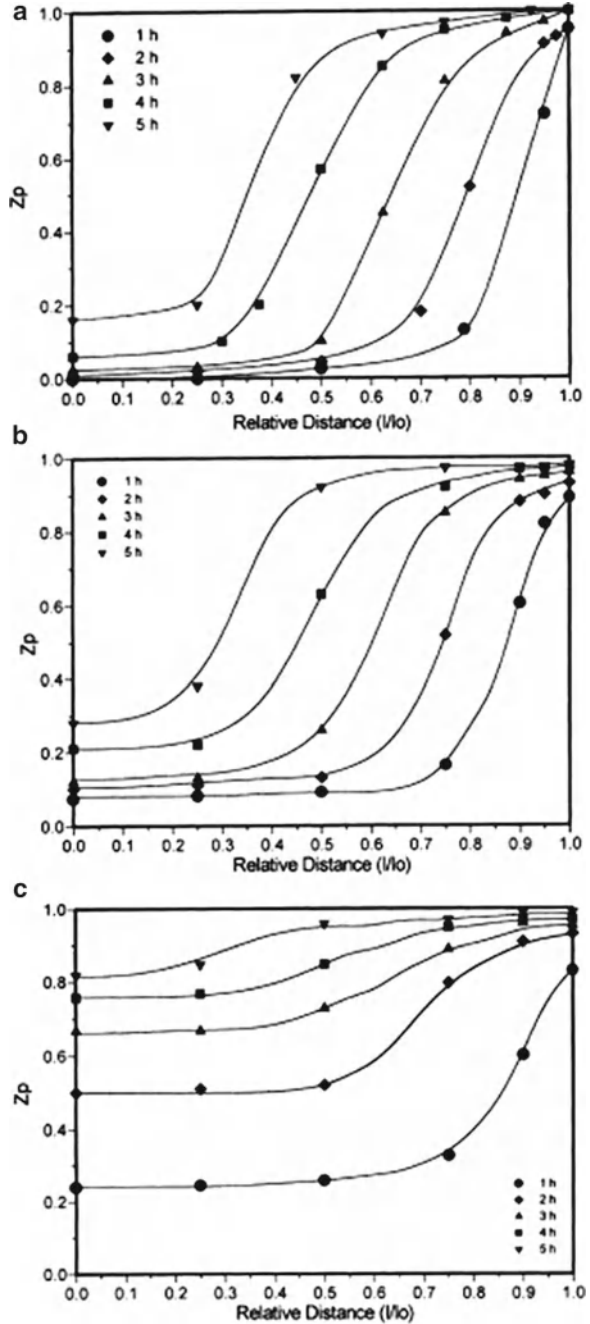


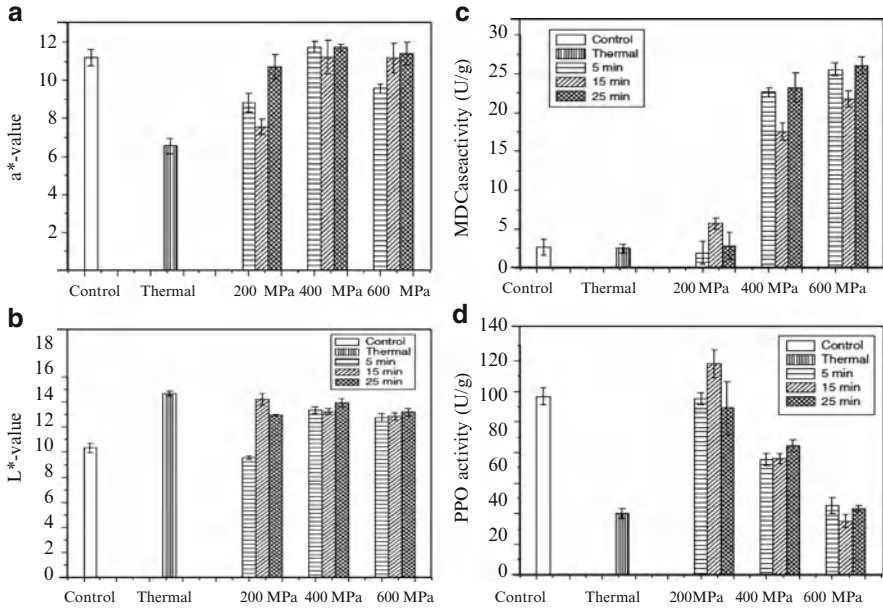


**Fig. 2.23** Effect of thermal blanching and pressure treatments (a) on ascorbic acid content, (b, c) on the firmness of bell peppers. Different letters indicate cases of major effects. *C* refers to unprocessed sample, *BI.1* and *BI.2* refer to blanching at 70 °C for 1 min and 2.5 min, respectively, *BII.1* and *BII.2* refer to blanching at 80 °C for 1 and 2.5 min, respectively, *BIII.1* and *BIII.2* refer to blanching at 98 °C for 1 and 2.5 min, respectively, *PI.1* and *PI.2* refer to pressurization at 100 MPa for 10 and 20 min, respectively, *PII.1* and *PII.2* refer to pressurization at 200 MPa for 10 and 20 min, respectively (From Castro, S. M., Saraiva, J.A., Lopes Da Silva, J. A. et al. 2008. *Food Chem.* 107: 1436–1449. With permission)

Pressure-shift-freezing (400 MPa) resulted in preservation of textural properties even after freezing due to reduced membrane damage (Luscher et al. 2005). Optimization of the high-pressure/low-temperature process (freezing and thawing) for whole potato resulted in better quality and safety, improvement in color, and reduction in drip loss during thawing (Benet et al. 2006).

**Fig. 2.24** Distribution of cell disintegration index with respect to distance from the center of the potato samples (thickness 10 mm) during osmotic dehydration of: (a) control sample; (b) pressure pretreated at 200 MPa, and (c) 400 MPa for 10 min (Rastogi, N.K., Angersbach, A., and Knorr, D. 2000c. *J. Food Eng.* 45: 25–31. With permission)





**Fig. 2.25** Effects of high pressure on (a, b) instrumental color parameters, (c) MDCase activity, and (d) PPO activity of spinach puree (From Wang, R., Wang, T., Zheng, Q., Hu, X., Zhang, Y., Liao, X. 2012. *J. Sci. Food Agric.* 92: 1417–1423. With permission)

### 2.2.17 Spinach

HPT (400 MPa, 30 min, 5 °C) extensively effected the structure of spinach. The soft parenchyma cells of spinach leaves were completely destroyed by pressure treatment (Prestamo and Arroyo 1998), while the retention of visual green color and chlorophyll contents of HP-treated (200–600 MPa, 5–25 min) spinach puree was quite high. The activities of chlorophyllase (chlorophyll degradation enzyme) and PPO (responsible for enzymatic browning) were decreased by pressure treatment, while POD showed higher resistance to pressure. But the activity of Mg-dechelataase was dramatically increased after HPT (Fig. 2.25, Wang et al. 2012a, b).

### 2.2.18 Tomatoes

The exposure of whole cherry tomatoes to HPT up to 400 MPa resulted in decrease in hardness due to action of polygalacturonase (PG) enzyme, which hydrolyzed low methoxypectin to water-soluble galacturonic acid. Further, increase in pressure up to 500 or 600 MPa showed less decrease in hardness (or % cell rupture), and

tomatoes appeared similar to controls. The increase in firmness was attributed to the action of PME to produce low methoxypectin, which formed a gel network with divalent ions leading to tissue hardening (Tangwongchai et al. 2000). Kuo (2008) showed that HPT (200–500 MPa) of tomatoes retains color, extractable total carotenoids, lycopene content, and antioxidant activity. The residual activities of PME and PG were in the lower range of pressure (200 and 400 MPa for PME and PG, respectively), whereas these activities were higher for higher pressure (500 MPa). Viljanen et al. (2011) indicated that HPT (800 MPa, 20 °C) decreased the levels of certain volatiles, namely aldehydes, ketones, and alcohols present in tomatoes, whereas the levels of hexanal, heptanal, and octanal increased. Processing at 800 MPa and 60 °C could not preserve fresh tomato odor, but resulted in a marked increase in the intensity of cooked-tomato and tea-like odor.

Water-insoluble antioxidants, lycopene, and  $\beta$ -carotene did not change as a result of high pressure to tomato puree, but structural changes in tissue resulted in a decrease in recovery of carotenoids and increase in water-binding capacity. However, antioxidant levels of the water-soluble fraction increased after storage at 4 °C for 21 days (Fernandez et al. 2001b). HPT of tomatoes neither had a significant impact on  $\beta$ -carotene nor on antimutagenicity (Butz et al. 2002). High pressure (400 MPa) along with citric acid and NaCl are used for producing minimally processed tomato products with optimal sensory and microbiological characteristics, having resulted in 4-log reduction of total microbial counts along with a significant inactivation of PPO, POD, and PME (Plaza et al. 2003).

Sanchez et al. (2004, 2006) showed that HPT (200 MPa) affects the structure of cellular tomato matrix in such a way that various carotenes are released differently on the basis of their chemical features and chromoplast location. The treatment (400 MPa, 25 °C, 15 min) of tomato puree resulted in higher redness, carotenoids, and vitamin C than for the case of pasteurization at low temperature (70 °C, 30 s) or high temperature (90 °C, 1 min). McInerney et al. (2007) indicated that antioxidant capacity and total carotenoid content are not affected by HPT. Hsu et al. (2008) found that HPT (500 MPa) of tomato juice resulted in inactivation of microorganisms and pectolytic enzymes, improvement in the extractable carotenoids and lycopene contents, and retention of vitamin C compared with fresh juice.

Qiu et al. (2006) indicated that the highest stability of lycopene in tomato puree was obtained by HPT at 500 MPa and further storage at 4 °C. Varma et al. (2010) indicated that pressure can cause conformational change in lycopene from the all-trans to *cis* isomer form. HPT (320–620 MPa for 3 min) of lycopene isomers in both of the two systems tributyrin (model system) and tomato homogenate (real food system) showed an increase in *cis* isomer content compared to the control.

Krebbbers et al. (2003) demonstrated that HPT (300–500 MPa, 20–90 °C) of tomato puree at ambient temperature is a suitable alternative to conventional processing techniques without causing marked losses in color and sensory properties at up to 8 weeks of storage at 4 °C. Rodrigo et al. (2007) found that combined thermal and HPT (300–700 MPa, 65 °C) did not result in visual color degradation of tomato puree. Dede et al. (2007) indicated that HPT (150–250 MPa) of tomato juices leads to higher retention of ascorbic acid, antioxidant activity, and minimum color loss at

up to 1 month of storage at 4 °C or 25 °C. Patras et al. (2009b) reported that high-pressure-processed (600 MPa) tomato puree results in retention of more than 90 % of ascorbic acid compared to thermally processed samples, but phenolic contents were unaffected by thermal or high-pressure treatments.

The combined pressure/temperature treatment was shown to be an attractive alternative to thermal sterilization for preserving tomato juice quality. Pressure-assisted thermal processing (600 MPa, 100 °C, 10 min) and HPT (700 MPa, 45 °C, 10 min) significantly improved the extractability of lycopene over thermal processing (100 °C, 35 min). Processed samples were stored at 4 °C, 25 °C, and 37 °C for up to 52 weeks. All-*trans* lycopene was found to be fairly stable to isomerization during processing, and the *cis* isomer content of the control and processed juice did not differ significantly (Gupta et al. 2010). Significant degradation of all-*trans*  $\beta$ -carotene occurred as a function of pressure, temperature, and time. Its retention in processed samples was found to vary between 60 and 95 %. The in-vitro bioaccessibility of carotenoids was not significantly different for all the treatments (Gupta et al. 2011).

### **2.2.19 Turnip**

Ueno et al. (2009) reports that HPT (400 and 600 MPa) of turnips causes a unique green-blue color due to biochemical changes during storage for 7 days at 4 °C. The mechanism of green-blue compound formation apparently resulted from a biochemical pathway for green-blue pigment synthesis containing oxygen-dependent steps and possibly enzymatic reactions.

## **2.3 Other Plant Products**

### **2.3.1 Aloe Vera**

Galvez et al. (2011) indicated that high-pressure (300–500 MPa, 1–5 min), blanching, enzymatic, and microwave pretreatments increases the water diffusion coefficient compared to control samples during dehydration of Aloe vera gel. Microwaves followed by high pressure resulted in fastest drying rates and increased firmness. High pressure in combination with convective drying produced dried aloe with high antioxidant attributes. Further, Galvez et al. (2012) found that HPT (400 and 500 MPa) leads to undetectable levels of microorganism counts and higher antioxidant activities, as well as vitamin C and E levels. Navarrete et al. (2012) reported that HPT (300–500 MPa) of Aloe vera suspension during storage at 4 °C exhibits shear-thinning behavior. The treatment did not modify gel properties, but influenced the rheological properties.

### 2.3.2 *Green Tea*

High pressure extraction appears to have a great potential for extracting caffeine from green tea leaves. A comparison of ambient temperature extraction, ultrasonic extraction, and heat reflux extraction showed that high-pressure-assisted extraction resulted in higher yields, shorter extraction times, and lower energy consumption. The highest yield of caffeine (4.0 %) was obtained at 50 % ethanol concentration, liquid/solid ratio of 20:1, and 500 MPa pressure applied for 1 min (Fig 2.26a, b, Jun 2009). Later, Jun et al. (2011a) indicated that total phenolic contents and the antioxidant activities increase with an increase in applied pressure up to 450 MPa (Fig 2.26c). Further, Jun et al. (2011b), on examination of microstructure, revealed that high pressure could result in the disruption of leaf tissue, cellular walls, membranes, and organelles leading to enhanced transfer of solvents into the leaf material and the soluble constituents (active compounds) into the solvents.

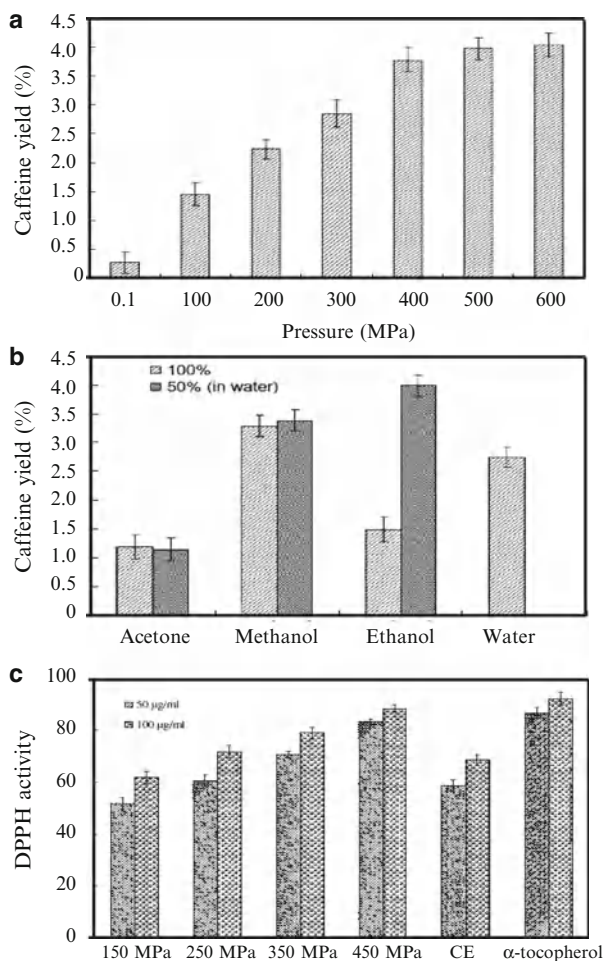
### 2.3.3 *Herbs and Roots*

Pennywort herb juice is a nutraceutical drink considered to provide health benefits. Therefore, to preserve all of its aroma and active components, a nonthermal process such as ultra-high pressure was found to be more appropriate than pasteurization and sterilization. In comparison with heated juices, high-pressure-treated samples retained more volatile compounds such as linalool and geraniol similar to those present in fresh juice, whereas some volatiles such as  $\alpha$ -terpinene and ketones were apparently formed by thermal treatment (Apichartsrangkoon et al. 2009).

High pressure extraction of salidroside from the herb *Sedum (Rhodiola) sachalinensis* at room temperature showed higher efficiency (0.40 % in 3 min) as compared to that of ultrasonic extraction (0.29 % in 30 min) and reflux extraction (0.30 % in 120 min) (Bi et al. 2009).

Clariana et al. (2011a) studied the effects of combined pressure/temperature treatments (200–600 MPa, 20 °C and 40 °C) on the color, texture, antioxidant activity, and glucosinolate profile of fresh-cut swede roots (*Brassica napus* var. *napobrassica*) as an alternative to traditional blanching techniques. All the studied treatments resulted in a loss of hardness, water binding capacity, and loss of antioxidant capacity. The strongest alteration of texture was observed at 400 MPa, while 600 MPa better preserved the texture properties. Blanching caused less total color changes than HPT.

Application of combined high pressure and microwave extraction of ginsenosides from *Panax ginseng* resulted in higher yields than other extraction methods, including soxhlet extraction, ultrasound-assisted extraction, and heat reflux extraction. The technique not only took a shorter time but also afforded higher extraction yields of ginsenosides (Yutang et al. 2008). High-pressure extraction of fresh and red ginseng was found to be more effective than thermal extraction (Lee et al. 2011).



**Fig. 2.26** Effect of (a) pressure, and (b) different solvents on the extraction yields of caffeine. (c) Comparison of DPPH activity of  $\alpha$ -tocopherol and GTE collected by high-pressure extraction at two different concentrations (50 and 100  $\mu$ g/ml). CE stands for conventional extraction (From Jun, X. 2009. *J. Food Eng.* 94(1): 105–109, Jun, X., Deji, S., Ye, L., Rui, Z. 2011a. *Food Res. Intl.* 44(9): 2783–2787. With permission)

### 2.3.4 Jam

Powdered sugar, pectin, citric acid, and freeze-concentrated strawberry juice was mixed, degassed, and then pressurized (400 MPa, 5 min) to form jam. The product had a bright red color and retained all the original flavor compounds (Watanabe et al. 1991). Kimura et al. (1994) have shown that pressure-treated jam had better



quality than heat-treated jam. The pressure-treated jam could be stored at refrigeration temperature up to 3 months. High-pressure-processed jam samples had better retention of anthocyanins (pelargonidin-3-rutinoside and pelargonidin-3-glucoside) (Gimenez et al. 2001). The increase in pectin concentration in high-pressure-processed (400 MPa, 5 min) strawberry jam resulted in increase in storage and loss moduli and decrease in absorbance intensity (Dervisi et al. 2001). A combined osmotic dehydration and HPT (550–700 MPa, 45–75 °C) process for grapefruit jam preservation resulted in only partial inactivation of PME and POD due to presence of pressure-stable fractions. The antioxidant capacity was not affected by the treatment (Igual et al. 2013).

### 2.3.5 Smoothies

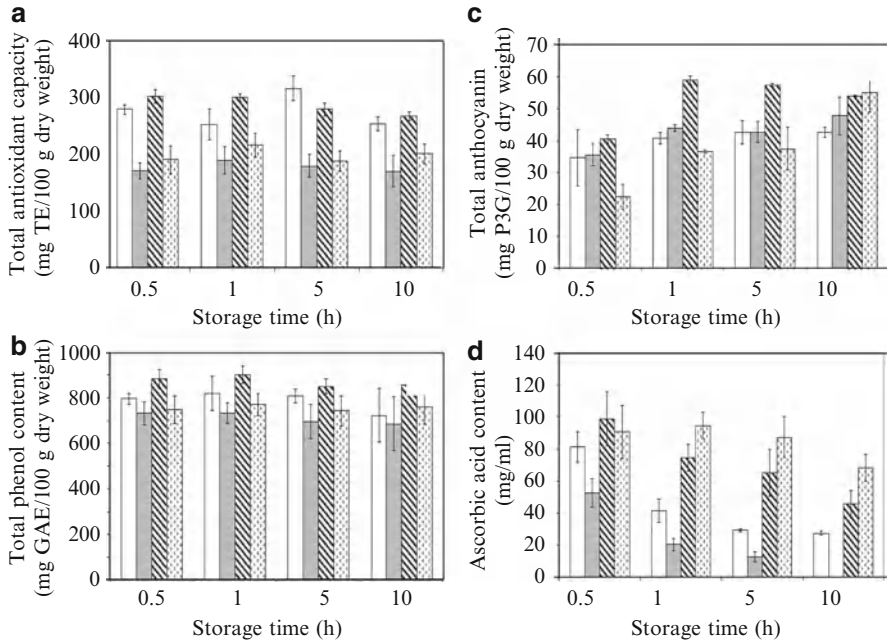
Fruit smoothies have become popular with consumers and may significantly contribute to daily antioxidant intakes. HPT can help retain antioxidants in fruit smoothies offering a unique selling point for processors.

Keenan et al. (2010) demonstrated that compared to HPT (450 MPa, 1–5 min), thermal treatment ( $P_{70} \geq 10$  min) resulted in significant reductions in antioxidant activity and phenolic content. The redness of HPP smoothies increased compared to the fresh one. Storage also had a significant effect on color variables but the effect was more pronounced in high-pressure-treated samples stored for 30 days. Keenan et al. (2012) compared thermally ( $P_{70} \geq 10$  min) or high-pressure-processed (450 MPa/20 °C/5 min or 600 MPa/20 °C/10 min) fruit smoothie samples over a storage period of 10 h at 4 °C. The levels of total antioxidant, phenols, and anthocyanin content for the sample processed at 450 MPa were higher compared to the sample processed at 600 MPa. Ascorbic acid content degraded over the storage for all smoothies (Fig 2.27). Keenan et al. (2011) indicated that HPT (450 MPa) for 1 and 2 min resulted in higher levels of phenolic compounds (procyanidin B1 and hesperidin) than the sample processed for 5 min. Levels of flavanones and hydroxycinnamic acid were found to decrease after 30 days of storage at 2–4 °C.

### 2.3.6 Vegetable Beverages

The results indicated that the Mediterranean vegetable soup *gazpacho* subjected to treatment at 150 MPa led to better retention of carotenoids and antioxidative activity as compared to treatment at 300 MPa when stored at 4 °C for 40 days (Plaza et al. 2006b). Barba et al. (2010) indicated that HPT (100-MPa, 120–540 s) of vegetable beverage containing tomato, green pepper, green celery, onion, carrot, lemon, and olive oil retained more ascorbic acid compared to thermal treatment (90–98 °C, 15–21 s), whereas, color changes were less and no marked changes in total phenols for pressurized beverage compared to thermally treated samples was observed.





**Fig. 2.27** (a) Antioxidant capacity, (b) phenolic content, (c) anthocyanin content, and (d) ascorbic acid content of fresh (□) fruit smoothies and their thermal (■) and 450 (▧) and 600 (▨) MPa high-pressure-treated counterparts over 10 h storage at 4 °C (Keenan, D.F., Roszle, C., Gormley, R., Butler, F., Brunton, N.P. 2012. *Lebensm. Wiss. Technol.* 45(1): 50–57. With permission)

Zhao et al. (2012) demonstrated that nisin (100 IU/ml) with HPT (400 MPa/4 min or 500 MPa/2 min) or thermal pasteurization (85 °C/15 s) had a synergistic effect on the inactivation of total aerobic bacteria. The retention of the quality attributes namely chlorophyll a and b, color, lipoxigenase activity, and key odorants in cucumber juice drinks was significantly better in the high-pressure-treated samples than in the thermally pasteurized samples during 50 days of storage at 4°C. Besides, yeast and molds were completely inactivated by all treatments. It was indicated that HPT could be used to establish safety criteria for the commercial production of high-quality cucumber juice beverages.

# Chapter 3

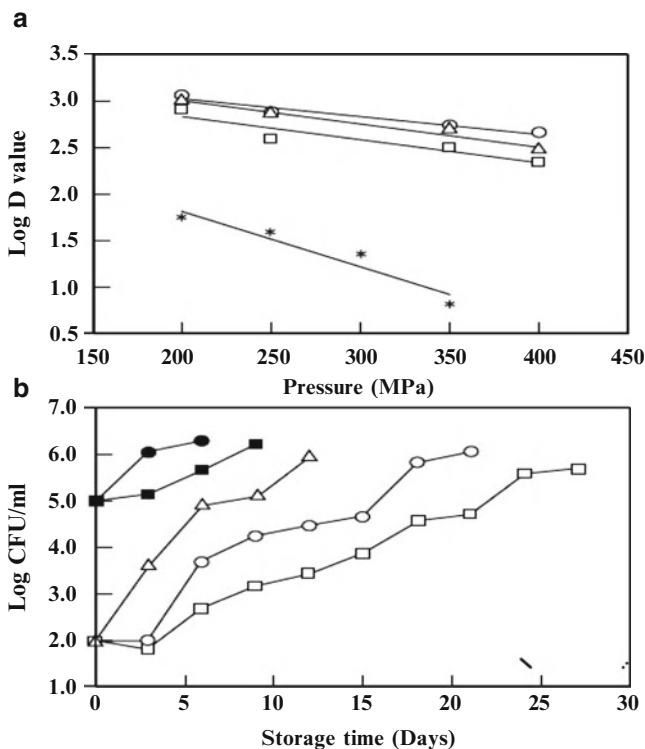
## High-Pressure Processing of Dairy Products

### 3.1 Milk

#### 3.1.1 Pasteurization

Research into the application of HPP for milk preservation began when Hite (1899) demonstrated that the shelf life of milk and other food products could be extended by pressure treatment. High pressure was found to be equally effective in destroying pathogenic and spoilage microorganisms compared to heat treatment. A number of researchers have studied inactivation of microorganisms (such as *Listeria monocytogenes*, *Staphylococcus aureus*, or *Listeria innocua*) either naturally present or introduced in milk (Erkman and Karatas 1997; Gervila et al. 1997). Periodic oscillation of high pressure was very effective for the destruction of pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis*, and this offers a promising alternative for the cold pasteurization of milk (Vachon et al. 2002).

Higher pressures resulted in higher rates of microbial destruction, enzyme alkaline phosphatase inactivation, as well as color and flavor changes as indicated by the associated lower D-values. Further, the rate of microbial destruction was much more rapid than enzyme inactivation or color and viscosity changes. Milk subjected to a microbial 4D high pressure process at 350 MPa had a shelf life of 25 days at 0 °C, 18 days at 5 °C, and 12 days at 10 °C (Fig. 3.1, Mussa and Ramaswamy 1997). HPT (400 MPa for 15 min or 500 MPa for 3 min) of thermally pasteurized milk increased shelf life by 10 days (Rademacher and Kessler 1997). A mild heat treatment (37 °C, 240 min or 50 °C, 10 min) inhibited the recovery of *Listeria monocytogenes* in high-pressure-processed milk, and the product was safely stored for 70 days at 25 °C (Koseki et al. 2008). Raw milk pressurized at 400 MPa for 30 min at 25 °C contained < 7 log psychrotrophs/ml after storage for 45 days at 7 °C, whereas unpressurized milk contained > 7 log of these bacteria after only 15 days (Garcia-Risco et al. 1998). Application of higher pressures for longer holding time at lower temperature resulted in greater destruction of indigenous microflora and *Escherichia*

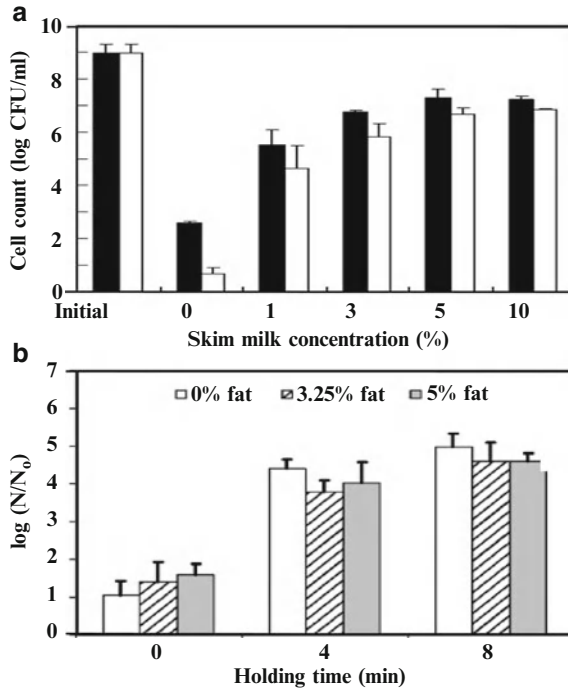


**Fig. 3.1** (a) Decimal reduction time curves for microorganisms (\*), color (○), alkaline phosphatase (Δ), and viscosity (increase; □) in milk subjected to ultra-high pressures (b) standard plate counts of high-pressure-processed milk (4D process 350 MPa) during storage at 0 °C (□), 5 °C (○), and 10 °C (Δ). Also shown are SPC of control samples stored at 0 °C (■) and 5 °C (●) (From Mussa, D.M., and Ramaswamy, H.S. 1997. *Lebensm. Wiss. Technol.* 30: 551–557. With permission)

*coli* in raw milk (Pandey et al. 2003). The Weibull model described the high pressure (400–600 MPa, 22 °C) inactivation kinetics of *Escherichia coli* and *Listeria innocua* (Buzrul et al. 2008b).

The protein fractions of skimmed milk provided protection against the injury and inactivation of *Escherichia coli* during HPT (Fig. 3.2a, Narisawa et al. 2008). Casein and lactose present in milk provided the major baro-protection effect to *Escherichia coli* in milk during HPT. Fat content in milk (0–5 %) had no significant effect on the destruction (Fig. 3.2b, Ramaswamy et al. 2009).

The gram-negative bacteria, in this case, were found to be more sensitive to high pressure, either alone or in combination with nisin, than gram-positive bacteria (Black et al. 2005). Later, Black et al. (2008) showed that combinations of HPT and nisin resulted in high levels of germination of *Bacillus* spores, but complete inactivation was not achieved. Further, the combination of high pressure with a bacteriocin (lactacin) was shown as a promising and natural method for increasing the

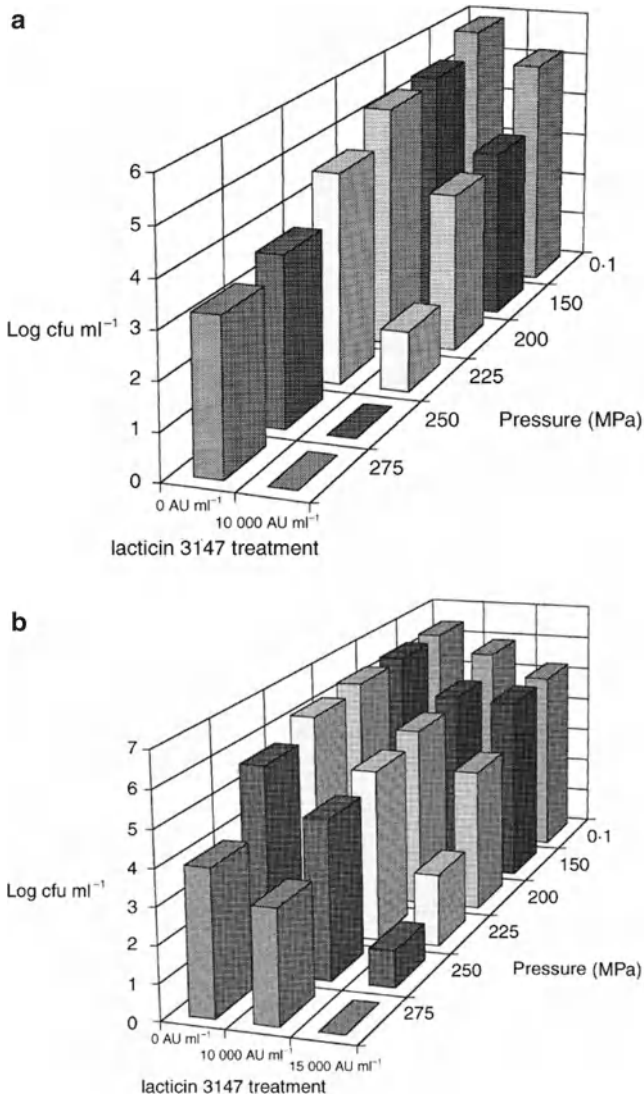


**Fig. 3.2** (a) Effect of skimmed milk concentration on the surviving and uninjured cells of *Escherichia coli* by HPT at 25 °C, 250 MPa for 20 min. Black bars indicate the surviving cells and white bars indicate the uninjured cells. (b) A comparison of logarithmic cycle reduction of *E. coli* K12 in 400 MPa treated milk with different fat contents (From Narisawa, N., Furukawa, S., Kawarai, T., Ohishi, K., Kanda, S., Kimijima, K., Negishi, S., Ogihara, H., and Yamasaki, M. 2008, *Intl. J. Food Microbiol.* 124: 103–107; Ramaswamy, H.S., Jin, H., and Zhu, S. 2009. *Food Bioprod. Proc.* 87: 1–6. With permission)

efficiency and safety of HPP of milk. It resulted in a synergistic effect in controlling microbial flora of milk without significantly influencing its cheese-making properties (Fig. 3.3, Morgan et al. 2000). Other antimicrobial peptides such as lactoferrin and lactoferricin (500 µg/ml) in combination with high pressure (155–400 MPa) also resulted in enhanced microbial inactivation (Masschalck et al. 2001).

The divalent cations  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  protect bacteria against HP-induced inactivation because of their stabilizing effect on the cell membrane. The buffering capacity of dissociated anions, i.e., phosphate and citrate, counteracts the HP-induced decrease in pH observed in milk, which would otherwise render bacteria more susceptible to HP-induced inactivation (Huppertz et al. 2005). HPT (400 MPa, 30 min) of milk did not result in a significant variation in the content of  $\text{B}_1$  and  $\text{B}_6$  vitamins (pyridoxamine and pyridoxal) (Sierra et al. 2000).

Ramaswamy et al. (2010) studied the destruction kinetics of *Clostridium sporogenes* inoculated in milk subjected to different pressure, temperature, and time



**Fig. 3.3** The effect of HP and lacticin 3147 on (a) *Staphylococcus aureus* ATCC6538 (b) *Listeria innocua* DPC1770 viability (From Morgan, S.M., Ross, R.P., Beresford, T., and Hill, C. 2000. *J. Appl. Microbiol.* 88: 414–420. With permission)

combination treatments (700–900 MPa, 80–100 °C). Higher pressures and higher temperatures resulted in a higher destruction rate and a higher microbial count reduction. Vazquez et al. (2006, 2007) indicated that combination of high pressure with temperature for the processing of milk promoted the formation of few compounds leading to generation of ‘cooked’ milk flavor and sensory acceptance of treated milk was not very high.

Huppertz et al. (2005) reviewed the effect of HP on the range of bacteria naturally present (total microflora, aerobes, aerobic mesophiles, psychrotrophs, coliforms) and exogenously added (*Escherichia coli*, *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, and *Salmonella enteritidis* etc.) in milk. Further, Trujillo et al. (2002); Huppertz et al. (2002, 2005), and Chawala et al. (2010) reviewed the effect of HP on properties and contents of milk.

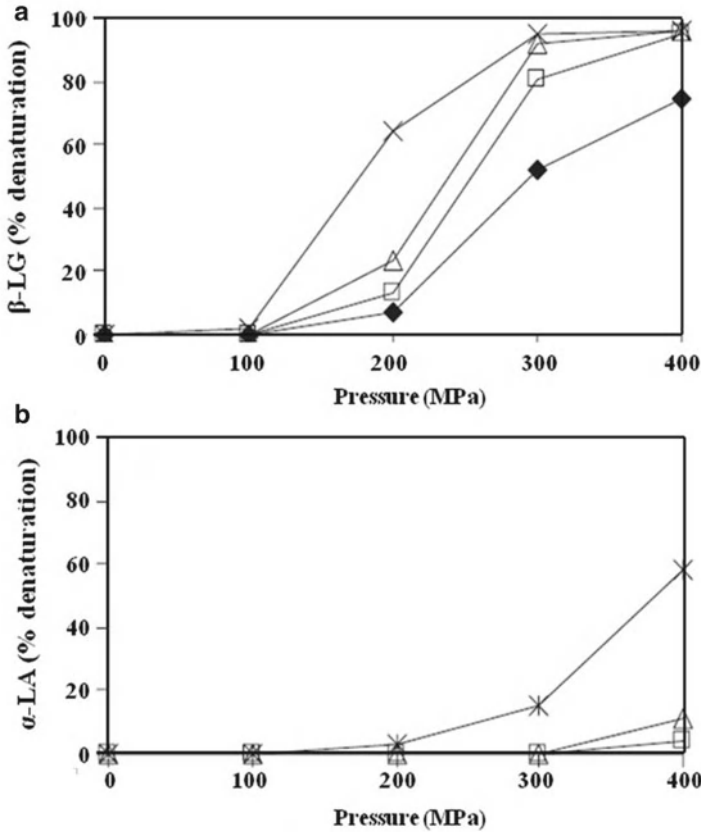
### 3.1.2 *Whey Protein*

High pressure up to 300 MPa did not result in significant decrease in  $\beta$ -lactoglobulin in whey, whereas further increase in pressure resulted in decreased  $\beta$ -lactoglobulin (Pandey and Ramaswamy 1998; Brooker et al. 1998). HPT (200–600 MPa) prior to enzymatic hydrolysis of whey protein concentrate with proteinase led to a decrease in  $\beta$ -lactoglobulin, but  $\alpha$ -lactalbumin did not change, whereas heat treatment in place of HPT resulted in decrease in both the proteins (Nakamura et al. 1993). The decrease in  $\beta$ -lactoglobulin was attributed to the exposure of side chains of buried amino acids to solvent (Alvarez et al. 2007). Denaturation of  $\beta$ -lactoglobulin took place at pressures as low as 200 MPa and the extent of which was found to increase with an increase in holding time and treatment pressure. Also,  $\alpha$ -lactalbumin was denatured only at pressures 400 MPa, and no effect of milk solids concentration was observed (Anema 2008a, b). A pressure treatment of 500 MPa at 25 °C denatures lactoglobulin, whereas denaturation of immunoglobulins and lactalbumins occurs only at the highest pressures, particularly at temperatures above 50 °C (Felipe et al. 1997). An increase in the temperature of the HPT up to 60 °C did not induce  $\beta$ -lactoglobulin denaturation at 100 MPa, but at higher pressures denaturation increased with increasing temperature. At the same time, almost 60 % of  $\alpha$ -lactalbumin was denatured by treatment at 400 MPa and 60 °C (Fig. 3.4, Lopez-Fandino and Olano 1998).

High pressure (100–300 MPa) combined with selected food-grade proteinases can be used as a treatment to remove the antigenicity of whey protein hydrolysate enabling its use as ingredients of hypoallergenic infant formulas (Penas et al. 2006). Pepsin and chymotrypsin under high pressure (400 MPa) produced hydrolysates in which  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin were totally proteolyzed resulting in large and hydrophobic peptides. Such hydrolysates showed reduced antigenicity, human IgE-binding properties, improved heat stability, and superior emulsion activity (Chicon et al. 2009). HPT of whey protein concentrate increased the number of binding sites which led to certain modifications in proteins, enhanced hydrophobicity, and showed promising results for improving functional properties of foods (Liu et al. 2005).

### 3.1.3 *Milk Enzymes*

Milk enzymes were much less sensitive to pressure. Only alkaline phosphatase and proteinases were completely inactivated at 1,000 MPa. A small increase in the

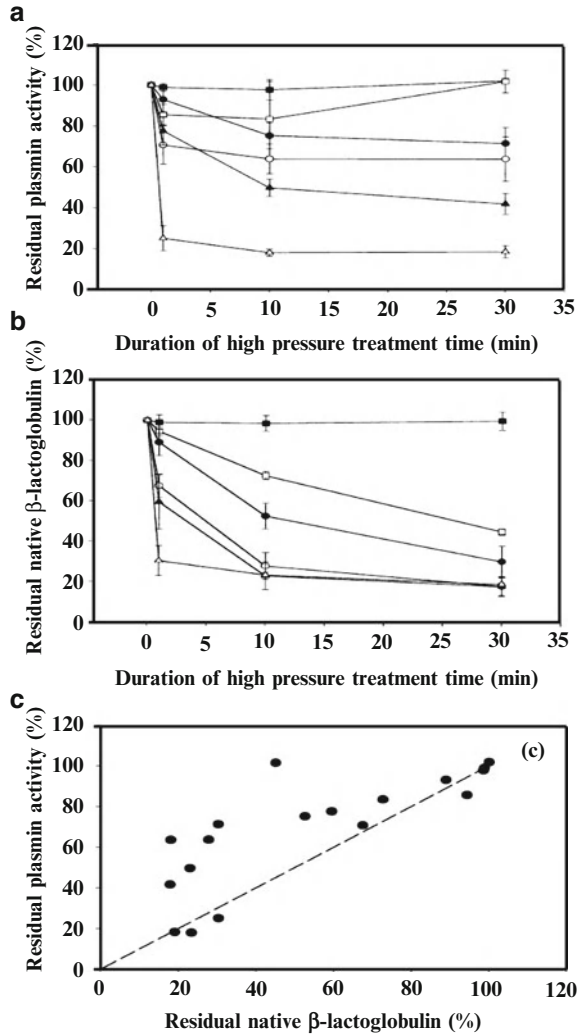


**Fig. 3.4** (a) Effect of pressurization on milk at 25 °C (♦), 40 °C (□), 50 °C (Δ), and 60 °C (×) for 15 min on the percentage of denaturation of (a) β-LG (β-lactoglobulin) and (b) α-LA (α-lactalbumin) (From Lopez-Fandino, R., and Olano, A. 1998. *Intl. Dairy J.* 8: 623–627. With permission)

lactoperoxidase activity due to antagonistic effect of HP and temperature was noted, which indicated that pressure treatment might lead to preservation of the lactoperoxidase activity, thereby maintaining the antimicrobial potency of milk (Ludikhuyze et al. 2001). HPT for a short time was reported to enhance activity of lipoprotein lipase and glutamyl transferase of milk. But, long-time (100 min) pressure treatment did not bring about any inactivation of lipase, while glutamyl transferase followed first-order inactivation kinetics (Pandey and Ramaswamy 2004).

Felipe et al. (1997) showed that HPT (500 MPa, 10 min, 25 or 50 °C) did not change the activity of alkaline phosphatase in goats' milk. Xanthine oxidase was proposed as an indicator of the HPT of milk. It was resistant to high-pressure exposure at 400 MPa at 25 °C and, at higher pressures; it was inactivated following first-order kinetics (Olsen et al. 2004). Ludikhuyze et al. (2000) studied the effect of high pressure on alkaline phosphatase to use it as an indicator of HPT. It was concluded that alkaline phosphatase could be an indicator of the absence of nonsporegenic

**Fig. 3.5** Residual native (a) plasmin, (b)  $\beta$ -lactoglobulin concentrations in milk determined immediately after HPT at 50 MPa (■), 300 MPa (□), 400 MPa (●), 500 MPa (○), 600 MPa (▲), and 800 MPa (Δ), for a range of times, relative to untreated control milk, (c) plot of the relationship between residual plasmin activity (%) and native  $\beta$ -lactoglobulin (%) (From Scollard, P.G., Beresford, T.P., Needs, E.C., Murphy, P.M., and Kelly, A.L. 2000. *Intl. Dairy J.* 10: 835–841. With permission)



pathogens, but an acceptable level of residual activity should be adequately defined to avoid overprocessing.

Scollard et al. (2000) indicated that  $\beta$ -lactoglobulin was denatured and plasmin activity was decreased at pressures higher than 300 MPa and 400 MPa, respectively. The loss of activity was not well correlated with  $\beta$ -lactoglobulin denaturation (Fig. 3.5). HPT influenced proteolysis in milk. During the storage of treated milk, treatment at 50 MPa had little effect on proteolysis, but at 300–400 MPa proteolysis was increased, possibly due to changes in micelle structure facilitating increased availability of substrate bonds to plasmin; whereas after 500 MPa, the proteolysis during storage of milk was less than that observed in raw milk. Garcia-Risco et al. (2000) also demonstrated that HPP at higher temperatures considerably increased plasmin inactivation.



A synergistic effect of temperature and high pressure was observed in the range of 300–600 MPa, whereas an antagonistic effect was observed at 600 MPa, most likely due to stabilization of enzymes by disruption of disulfide bonds (Borda et al. 2004a, b). The combined effects of high pressure (300–600 MPa, 40–60 °C) and homogenization resulted in inactivation of protease activity in milk, which extended its shelf life (Sainz et al. 2009).

### **3.1.4 Casein**

HPT at 100–200 MPa had little influence on average casein micelle size at ambient temperature, whereas 250 MPa for more than 15 min increased the micelle size, and at a pressure higher than 300 MPa reduces micelle size by less than 50 %. HP-induced increase in micelle size at 250 MPa is greater after a longer treatment time and at a higher treatment temperature and higher milk pH, as well as when the original untreated micelles are larger. Increases in micelle size at 250 MPa are probably due to the formation of large aggregates from HP-disrupted casein micelles (Huppertz et al. 2008; Anema et al. 2005a). HP-induced disruption of casein micelles and dissociation of casein from the micelle were due to solubilization of micellar calcium phosphate, as well as the disruption of intramicellar hydrophobic and electrostatic interactions (Regnault et al. 2004).

HP-induced micellar dissociation resulted in the breaking of linkages between casein and inorganic constituents. Besides, HP also exerts a disruptive effect on hydrophobic interactions that allowed the loss of casein clusters, stabilized through colloidal calcium phosphate, from the casein micelle (Needs et al. 2000a). HPT of acidified milk ( $\text{pH} \leq 6.0$ ) led to intensive destruction of the colloidal structure and an increase in the content of nonsedimentable casein (Arias et al. 2000), whereas at neutral or alkaline pH the solubilization of colloidal calcium phosphate is limited, which helps to maintain the colloidal structure (Anema et al. 1997).

Initially, application of HP (250–300 MPa) led to a rapid micellar disruption, which was found to be reduced by cross-linking of the casein micelles through transglutaminase prior to pressure treatment (Huppertz and Smiddy 2008). Pressurization of milk in the range of 150–300 MPa favored the formation of a large number of small micelles that coexisted with a fraction of large micelles and appeared perfectly spherical with smooth and well-defined surfaces which originated due to secondary adsorption of casein (Knudsen and Skibsted 2010).

## **3.2 Cheese**

HP resulted in casein micelle disruption, whey protein denaturation, increase in milk pH and cheese yield, and reduction in rennet coagulation time, which indicates its significant potential in the cheese-making process (O'Reilly et al. 2001; San-Martin et al. 2006).

### 3.2.1 Shelf-Life Extension

HPP can be utilized as an effective tool to extend shelf life while maintaining the quality attributes of this product. HPP (400–500 MPa) of goat milk cheese (inoculated with  $10^8$  CFU/g) showed no surviving *Escherichia coli* even after 15, 30, or 60 days of storage at 2–4 °C (Capellas et al. 1996). Application of HP substantially reduced the microbial load in Cheddar cheese, with 400 MPa for 20 min at 20 °C being sufficient to reduce the numbers of viable *Escherichia coli* and *Penicillium roqueforti* by 7- and 6-log-unit cycles, respectively, and to reduce the levels of *Staphylococcus aureus* by 3-log-unit cycles (O'Reilly et al. 2000).

HPT (400–700 MPa) was effective in reducing *Listeria monocytogenes* in gorgonzola cheese rinds (Carminati et al. 2004) and Turkish white cheese (Evrendilek et al. 2008) without significantly changing its sensory properties. HPT also resulted in total reduction in molds, yeasts, and Enterobacteriaceae counts for the cheese samples produced from raw and pasteurized milk. HPT (500 MPa, 10 min) significantly reduced the level of *Listeria monocytogenes* in the raw milk and so allowed the production of safer nonthermally processed camembert-type soft cheese (Linton et al. 2008).

Delgado et al. (2011a) demonstrated that HPT increased the food safety of raw goats' milk cheeses without affecting the original aroma of the cheese. Arriagada et al. (2012) showed that cheese treated at 300 and 400 MPa and stored at 4 °C had a shelf life of 14 and 21 days, respectively, compared to 7 days in untreated cheese.

### 3.2.2 Rennet Coagulation

Rennet coagulation time was not dependant on the pressure in the lower range (< less than 150 MPa), whereas at higher pressures (200–400 MPa) it decreased (Needs et al. 2000a). The decrease was due to HP-induced association of whey proteins with casein micelles. Further, increase in pressure (500–600 MPa) resulted in increased rennet coagulation time (Needs et al. 2000a). Rennet coagulation time of pressure-treated (500 MPa) milk was higher than pasteurized milk (72 °C, 15 s, Trujillo et al. 1999a).

HPT (400 MPa) of pasteurized milk resulted in decreased rennet coagulation time. At 600 MPa, the rennet coagulation time was found to decrease along with decrease in pH, initial counts of nonstarter lactic acid bacteria, protein and fat content. The treatment increased incorporation of  $\beta$ -lactoglobulin leading to increased yield (Voigt et al. 2010). Freshly prepared rennet-coagulated soft cheese subjected to HPT (291 MPa and 29 min) resulted in increased fat content (due to decrease in moisture), reduced lipid oxidation, acidity, and adhesiveness, whereas, hardness, and yellowness was found to increase (Okpala et al. 2010). Katsaros et al. (2010a) applied the protease actinidin (from *Actinidia chinensis*) as the milk clotting agent, and HP to control excessive proteolysis for the production of fresh

cheese without affecting the texture and sensory characteristics. Plant proteases can be a viable approach provided that excessive proteolysis after structure formation is regulated.

### 3.2.3 Yield

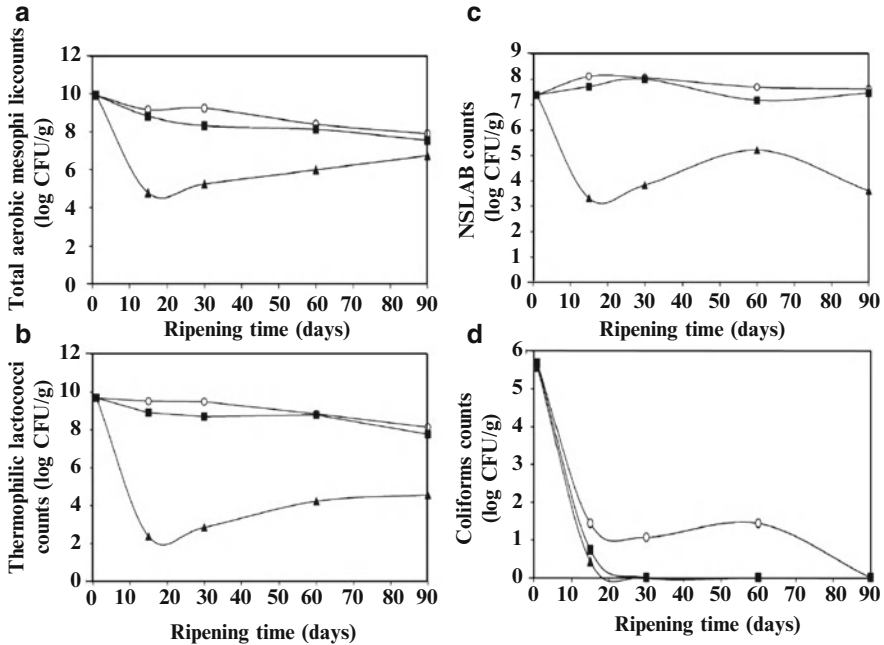
HPT induced denaturation of whey proteins and their association with casein, which resulted in increased cheese yield. HPT of milk may allow moisture to be trapped or held in cheese due to aggregation of casein molecules and fat globules leading to increased yield and higher moisture content of cheese (Drake et al. 1997). Reduced hardness of Cheddar cheese made from HP-treated milk was due to association of whey protein with casein in pressurized milk (Pandey and Ramaswamy 1998). The yield of cheese from HP-treated and subsequently heated milk was greater than that from unheated and unpressurized milk (Arias et al. 2000; Huppertz et al. 2005, 2008). Molina et al. (2000) refer to the increased yield of pressurization of pasteurized milk due to improvement in the coagulation properties of proteins. Alonso et al. (2011) reported on the suitability of frozen pressurized curd made from raw ovine milk for Hispanico cheese manufacture without altering its flavor characteristics but increased the yield of the ripe cheese.

### 3.2.4 Ripening

HP induces changes in biochemical processes such as glycolysis, lipolysis, and proteolysis during ripening of cheese leading to reduction in ripening time and quality improvement. The rate of ripening of commercial cheddar cheese due to HPT accelerated the degradation of  $\alpha_{S1}$ -casein and accumulation of  $\alpha_{S1}$ -1-casein (O'Reilly et al. 2001). HP-treated (500 MPa) goats' milk had higher pH and salt content, matured more quickly, and developed strong flavors (Trujillo et al. 1999b).

The odor of La Serena cheese during ripening made from raw Merino ewe's milk after the second day of HPT (300 or 400 MPa, 10 min) was scarcely affected; but after 50 days the volatile compound profile or the sensory characteristics were the same as those of the controls (Arques et al. 2007). Similarly, Juan et al. (2008) showed that HP (300 MPa, 10 min) on the day of manufacture resulted in decrease in  $\alpha_{S1}$ - and  $\beta$ -casein and increase in water-soluble nitrogen and free amino acids, which resulted in decrease in scores for taste, odor, and aroma quality compared to controls, whereas after 15 days of ripening the scores were similar to the controls. The samples had more homogeneous protein network, less crumbly texture, and highest percentage of short-chain fatty acids.

HPT (200 or 500 MPa, 15 min, 20 °C) on the 15th day of ripening of ovine brined cheese indicated that the treatment at 200 MPa did not affect the counts of total aerobic mesophilic bacteria, thermophilic lactococci, thermophilic lactobacilli, and non-starter lactic acid bacteria throughout ripening, whereas the treatment at 500 MPa



**Fig. 3.6** Counts of (a) total aerobic mesophilic bacteria, (b) thermophilic lactococci, (c) non-starter lactic acid bacteria (*NSLAB*), (d) coliforms in ovine brined cheese during ripening: untreated (-○-), HP-treated at 200 MPa (-■-) or at 500 MPa (-▲-) for 15 min (From Moschopoulou, E., Anisa, T., Katsaros, G., Taoukis, P., Moatsou, G. 2010. *Innov Food Sci Emerg* 11: 543–550. With permission)

resulted in significant reduction. Coliforms were reduced faster at nondetectable levels in HP-treated cheeses than in control cheese (Fig. 3.6, Moschopoulou et al. 2010).

Delgado et al. (2011b) studied the effect of HPT (400 or 600 MPa, 7 min) on volatile compounds applied to raw-milk goat cheese at different stages of ripening (1, 30, or 50 days). HPT applied at the beginning of maturation decreased the relative abundance of most volatile compounds, but enhanced the formation of ketones and other compounds. Changes were less intense when treatment was applied at the end of maturation. Camembert cheese manufactured from HP-treated (500 MPa, 10 min) bovine milk caused an increase in moisture content and decrease in protein content and plasmin activity. The treatment resulted in an altered cheese composition and ripening pattern, but with an acceptable sensory quality (Voigt et al. 2011). Besides, it resulted in the elimination of a number of risk factors, but the quality characteristics of the cheese were similar to the cheese made from untreated raw milk. The treatment resulted in increased proteolysis and higher levels of free fatty acids in cheese and the product was whiter, less hard, gummy, and chewy compared to control cheese (Voigt et al. 2012). HPT (50–400 MPa, 5–15 min) of white cheeses ripened in brine for 60 days indicated that the treatment did not affect moisture,

protein, and fat contents. A lower HP level (50 and 100 MPa) resulted in no changes in microstructures, while at higher levels (200 and 400 MPa) resulted in denser and more uniform structure (Koca et al. 2011).

### 3.3 Yogurt

HPT (200–300 MPa at 10–20 °C) of packaged yogurt neither modified the yogurt texture nor reduced the number of viable lactic acid bacteria; but it prevented the development of acidity. The pressure above 300 MPa resulted in overacidification and the number of viable lactic acid bacteria was reduced (Tanaka and Hatanaka 1992).

Acid-set gels prepared from HP-treated (100–600 MPa, up to 1 h) milk improved texture (rigidity and resistance to breaking) and syneresis resistance of the gels, which in turn resulted in viscosity improvement of yogurt-type products (Johnston et al. 1994). High water retention was only maintained in yogurts made from HP-treated milk and the firmness increased with an increase in pressure and the product was found to be stable during storage at 4 °C for 20 days (Ferragut et al. 2000). Lower values of fracture stress were observed in set yogurts made from milk treated at 600 MPa for 15 min compared to heat-treated milk (Needs et al. 2000b). The use of a mixture containing only 10 % of pressure-treated milk resulted in a creamy product that maintained the taste of conventional yogurt (Trujillo et al. 2002). Reys et al. (1999, 2001) showed that prolongation of the shelf life of yogurt by HPT can be obtained by complete inactivation of lactic acid bacteria.

HPT (550 MPa) of yogurt maintained desirable sensory characteristics longer than controls during storage for 4 weeks at refrigerated (4 °C) or room (20 °C) temperature. The pressure treatment prevented the postacidification of the product. The number of bacteria in the HP-treated yogurt stored at 4 °C was maintained at less than the therapeutic minimum level of  $10^6$  CFU/ml (Jankowska et al. 2005). The pressure treatment (550 MPa, 4 °C, 10 min) could be used to produce shelf-stable thicker and smoother fruit yogurt. No microbial spoilage took place in HP-processed sample even after 60 days of storage at 4.4 and 25 °C. Moreover, the count of lactic acid bacteria decreased to  $<10$  CFU/ml (Walker et al. 2006). Stirred yogurt made from reconstituted HP-treated (100–400 MPa, 25–90 °C, 10 min) skim milk prior to inoculation with yogurt culture showed that fermentation time was not affected by treatment. HPT of skim milk at lower temperature (25 °C) before or after heat treatment gave yogurts of similar viscosities to that of heat-treated milk, whereas lower viscosities were obtained when yogurts were made from HP-treated milk at elevated temperatures due to changes in interactions and structures of protein in the milk samples (Udabage et al. 2010).

A combination of transglutaminase and HPT of milk (when applied individually or in combination) proved to be an alternative treatment to produce yogurt with improved textural and sensorial characteristics. The samples made from HPT in combination with transglutaminase-treated milk exhibited higher firmness and

lower whey separation, while the sample made from HP-treated milk with or without subsequent transglutaminase treatment exhibited a creamier perception (Tsevdou et al. 2012).

### 3.4 Reconstituted Milk

HPT (up to 500 MPa) reduced the turbidity of reconstituted skim milk for all combinations of pH (5.5–7.5) and temperature (5–40 °C) due to micelle dissociation (Orlien et al. 2010). HP-treated reconstituted skim milk (200–600 MPa, 5–30 min) on acidification resulted in the formation of weak gel due to formation of restructured colloidal particles which were not stable to acidification because of the inability of redistributed  $\kappa$ -casein to stabilize these particles (Anema 2010).

HPT (200 MPa, 40 °C) applied to the milk protein concentrate before spray drying improved solubility (85 %) of the dried powder, which did not change even after 6 weeks storage at ambient temperature. The improved solubility was attributed to the altered surface composition arising from an increased concentration of nonmicellar casein in the milk due to HPT (Udabage et al. 2012).

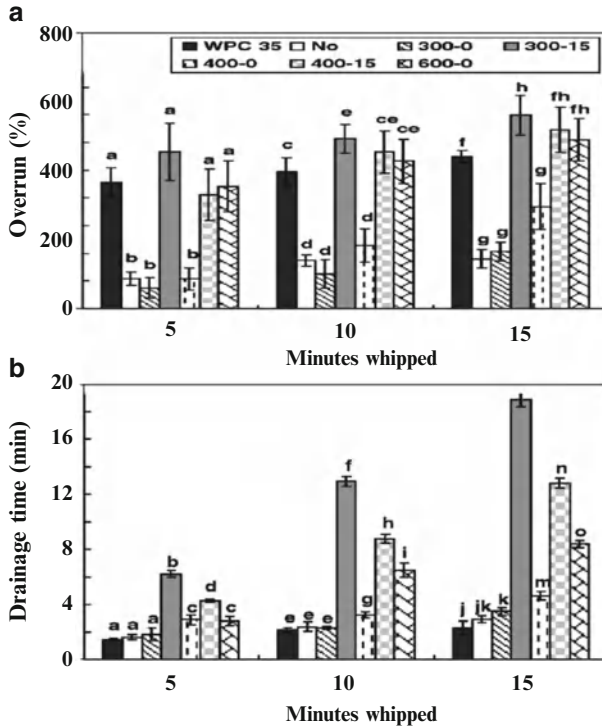
### 3.5 Ice Cream

HP (300 MPa, 15 min) enhanced the foaming properties of whey protein concentrate, which was added to low-fat ice cream to improve body and texture. Due to the impact of HP on the functional properties of whey proteins, the ice cream mix containing the whey protein exhibited an increased overrun and foam stability and hardness than ice cream produced with untreated whey protein (Fig. 3.7, Lim et al. 2008a, b).

### 3.6 Other Dairy Products

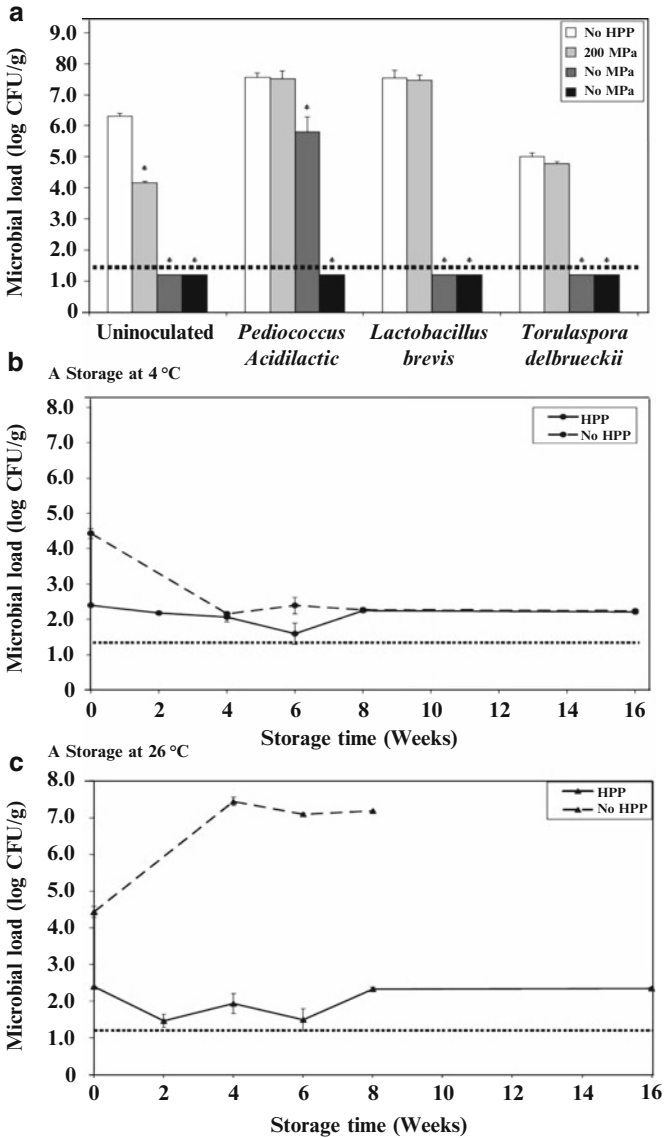
Waite et al. (2009) indicated that HPP (600 MPa, 3 min) of ranch dressing (combination of buttermilk, salt, garlic, onion, herbs, and spices, pH 4.4) resulted in decrease in *Pediococcus acidilactici* (the most pressure-resistant spoilage organism) by  $\geq 6.4$  log CFU/g. On the other hand, treatment at 600 MPa for 5 min prevented microbial spoilage throughout the storage period for 26 weeks at 4 °C and 26 °C without adverse changes in pH and emulsion stability (Fig. 3.8).

Sahu (2010) optimized the levels of HP (200–400 MPa), pressurization time (0–100 min), and coagulation temperature (30–70 °C) for the preparation of *chhana* (Indian cottage cheese). HPT at 280 MPa, pressurization time of 47 min, and coagulation temperature of 52 °C were found to be optimal for minimum lag, inflexion,



**Fig. 3.7** Effect of whipping time on (a) overrun and (b) drainage time (foam stability) (WPC35 commercial whey protein concentrate, No=Control, 300-0=300 MPa for 0 min, 300-15=300 MPa for 15 min, 400-0=400 MPa for 0 min, 400-15=400 MPa for 15 min, 600-0: 600 MPa for 0 min) (From Lim, S.Y., Swanson, B.G., Clark, S. 2008a. *J. Dairy Sci.* 91: 1299–1307. With permission)

and coagulation time of 0.0028, 5.19, and 3.87 min, respectively. Oh et al. (2009) showed that gelatinization of waxy rice starch in skim milk was retarded due to the presence of soluble milk minerals and lactose. Milk proteins (casein and whey protein) did not affect the degree of pressure-induced gelatinization. Al-Nabulsi et al. (2012) indicated that coagulant-induced milk gel produced by HPT (483 MPa) had higher storage modulus ( $G^*$ ) and firmer gel at cutting compared to heated milk. Increasing the pressure to 676 MPa caused a reduction in  $G^*$ , less firm gels, and an increase in milk turbidity.



**Fig. 3.8** (a) Efficacy of HPP (200–600 MPa, holding time 3 min) against natural microbiota of ranch dressing (uninoculated) and the inoculated spoilage bacteria, *Pediococcus acidilactici* OSY-JW1, *Lactobacillus brevis* OSY-JW1, and *Torulaspora delbrueckii* OSY-JW1. Dashed line indicates recovery method’s detection limit. (b, c) Microorganisms recovered on MRS agar from ranch dressing (pH 4.4) with or without HPT (600 MPa, 5 min) with extended storage at 4 °C or 26 °C (From Waite, J.G., Jones, J.M., Turek, E.J., Dunne, C.P., Wright, A.O., Yang, T.C.S., Beckwitt, R., Yousef, A.E. 2009. *J. Food Sci.* 74: M83–M93. With permission)



## Chapter 4

# High-Pressure Processing of Animal Products

High-pressure processing allows the decontamination of foods with minimal impact on their nutritional and sensory features. The use of HP to reduce microbial loads has shown great potential in the meat, poultry, and seafood industry. HP has proven to be a promising technology, and its industrial applications have grown rapidly, especially in the stabilization of ready-to-eat meats and cured products. The effect of HP on various animal products is presented in the following sections.

### 4.1 Beef

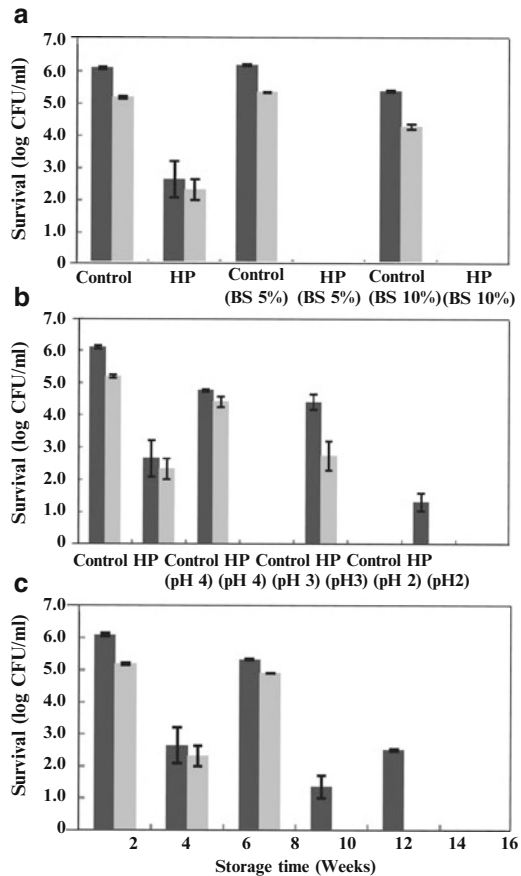
#### 4.1.1 Ground Beef

HPT (200–400 MPa) of fresh minced meat resulted in complete inactivation of *Pseudomonas fluorescens*, *Citrobacter freundii*, or *Listeria innocua*, whereas total flora was reduced up to 5 log cycles. The treatment led to the delay in microbial growth by 2–6 days during storage at 3 °C. Besides, it resulted in whitening because of globin denaturation and heme displacement or release (Carlez et al. 1993, 1995).

The textural properties of meat batters with walnuts were not affected by HPP; but hardness, cohesiveness, springiness, and chewiness of the cooked products were reduced by walnut addition (Ayo et al. 2005). Four cycles of short duration (400 MPa, 1 min) and single cycle of long duration (400 MPa, 20 min) led to almost similar reduction (4.38 log cycles) in microbial population in ground beef (Morales et al. 2008).

High-pressure sterilization of ground beef involving combination of HP with high temperature (700–900 MPa, 80–100 °C) took shorter time or lower temperature compared to conventional thermal processing (Zhu et al. 2008). HPP reduced *Escherichia coli* O157:H7 in ground beef by 3 log CFU/g and caused substantial sublethal injury resulting in further log reductions of bacteria during frozen storage.

**Fig. 4.1** Survival of *Escherichia coli* O157:H7 post-HPP and additional stresses, (a) bile salts (b), low pH, and (c) mild cooking conditions (From Black, E.P., Hirseisen, K.A., Hoover, D.G., Kniel, K.E. 2010. *J. Appl. Microbiol.* 108: 1352–1360. With permission)



The physiological status of the surviving *Escherichia coli* was affected by HP, sensitizing the cells to pH 3 and 4, bile salts at 5 % and 10 %, and mild cooking temperatures of 55–65 °C (Fig. 4.1, Black et al. 2010).

### 4.1.2 Beef Slices

HPT (100 MPa, 10–15 min) of beef slices within 24 h resulted in reduction in shear strength, loss of pink color, reduced loss of exudates, and higher score for cooked meat. However, after 7 days storage, shear strength of slices treated with HP was shown to be higher than the control sample (Jun et al. 1999). Air-blast freezing at (–30 °C) of HP-treated (650 MPa, 10 min) beef resulted in increased expressible moisture and no change in shear force. Color of the thawed sample was close to fresh sample. The stored sample (–18 °C, 45 days) recovered its original color after thawing, while microbial counts were below detection limits (Fernandez et al. 2007).

HPT (600 MPa, 20 °C, 3 min) reduced *Listeria monocytogenes* by more than 4 log CFU/g and extended the refrigerated shelf life of ready-to-eat meat. Besides, counts of aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria* spp., staphylococci, *Brochothrix thermosphacta*, coliforms, and fungi were also undetectable when stored at 4 °C for 98 days (Hayman et al. 2004). HPT (400 MPa, 10 min, 12 °C) of meat produced significant changes in the levels of volatile compounds due to microbial activity. Alcohol and aldehyde contents were decreased, whereas, other compounds, such as 2,3-butanedione and 2-butanone, were more abundant. Migration of branched-chain alkanes and benzene compounds from plastic packaging material was also observed (Canedo et al. 2009a).

A comparison of quality attributes of HPT (400–600 MPa, 35–55 °C) beef with nontreated and oven-cooked samples showed that TBARS-values of pressurized samples were lower than cooked samples after processing and throughout refrigerated storage. An increase in the ratio of omega-6 to omega-3 fatty acid ratio was found when pressure treatment was compared to raw samples, however, oven-cooked samples presented the highest ratio among all the treatments. HP altered the meat quality to a lesser extent than conventional cooking (McArdle et al. 2011).

### 4.1.3 Meat Tenderness

The tenderization of meat is mainly influenced by calpain and cathepsin enzymes. HP as well as ageing resulted in the reduction of calpain activity (Ouali 1990). HPT (up to 300 MPa) resulted in meat tenderization without heating due to increased myofibril fragmentation and modifications in its ultrastructure (Suzuki et al. 1992). Homma et al. (1995) demonstrated that total activity of calpains in pressure-treated muscle increased. It was attributed to the reduction in the levels of calpastatin, which in turn led to meat tenderization. Contrary to this, Qin et al. (2001) showed that HPT (100–300 MPa, 10 min) of beef resulted in decrease in total calpain activity, whereas the activities of acid phosphatase and alkaline phosphatase were not reduced.

In case of beef, HPT (520 MPa) did not improve tenderness or reduce the ageing period. Modification in myofibrillar components, reduction in sarcomere length, and increase in cooking losses resulted in increased toughness. The activity of cathepsin D and acid phosphatase increased due to breakdown of the lysosomes in the meat and enzyme activation (Jung et al. 2000). HP for short time (520 MPa, 260 s) reduced total flora and delayed microbial growth by 2 weeks. Discoloration of the meat occurred at pressure levels higher than 325 MPa, whereas, moderate pressure (130 MPa) improved meat color by increasing redness during 3 days of storage at 4 °C (Jung et al. 2003). Hardness of beef muscle increased with increasing pressure (200–800 MPa) at a constant temperature (up to 40 °C), whereas it decreased significantly with pressure treatment (200 MPa) at higher temperatures (60 °C or 70 °C). This probably results from accelerated proteolysis leading to loss of hardness (Han and Ledward 2004). HPT (200–600 MPa, 5 min, 5–7 °C) improved the digestibility of beef extracts and led to a decrease in allergenicity of the beef antigens (Han 2006; Han et al. 2006).

## 4.2 Pork

### 4.2.1 Dry-Cured Ham

HPT (400 MPa, 10 min, 12 °C) had a slight effect on the volatile fraction of Spanish dry-cured Serrano ham. Most of the compounds affected by HP originated from the metabolism of molds (Canedo et al. 2009b, c). Pressure-treated samples showed significantly higher levels of alcohols, aldehydes, and alkanes and lower levels of methylketones as compared to control samples (Canedo et al. 2009c).

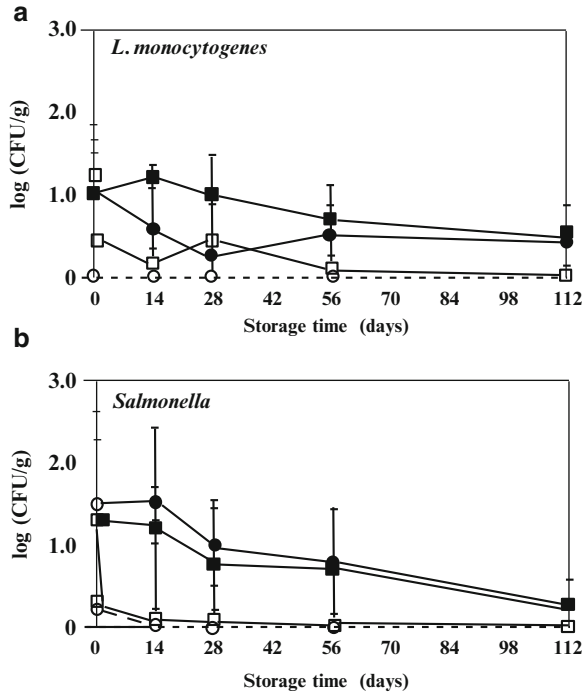
HPT (600 MPa) of sliced and vacuum-packaged commercial dry-cured ham during 50 days of refrigerated storage resulted in a reduction in the aerobic count and increase in lightness ( $L^*$  value). Besides, many sensory attributes were also modified resulting in an increase in hardness, chewiness, brightness, odor intensity, and saltiness (Clariana et al. 2011b). The treatment of sliced and vacuum-packaged commercial dry-cured ham at 900 MPa decreased superoxide dismutase and glutathione peroxidase activities and increased vitamin E content. In contrast, HPT at 400 MPa increased superoxide dismutase activity and showed no effect on vitamin E content and glutathione peroxidase activity. Fat, moisture, and collagen contents were not affected by HP. The treatment at 400 MPa increased the lightness ( $L^*$  values) and slightly modified the hardness, chewiness, saltiness, and color intensity (Clariana et al. 2012).

HPT (200–300 MPa, 15–30 min) of sliced vacuum-packed dry-cured Iberian ham during storage for 90 days at 4 °C showed a higher susceptibility to lipid oxidation than dry-cured loin. After 90 days of storage, lipid and protein oxidation increased while redness decreased in HP-treated and untreated dry-cured meat products (Cava et al. 2009). Similarly, Fuentes et al. (2010) also indicated that HPT (600 MPa) enhanced the oxidation of lipids and protein in vacuum-packaged Iberian dry-cured ham.

HPT (600 MPa, 5 min) of smoked dry-cured ham with NaCl resulted in elimination of *Listeria monocytogenes* and *Salmonella* after 14 days. However, these were present in NaCl-free until days 28 and 56, respectively, indicating that the NaCl-free processed dry-cured ham had lower stability than smoked dry-cured ham with NaCl (Fig. 4.2, Stollewerk et al. 2012a). Further, Stollewerk et al. (2012b) indicated that combination of fast drying process (QDS process) with HPT proved to be adequate for the production of safe NaCl-free processed dry-fermented sausages. Besides, the treatment assured absence of *Listeria monocytogenes* and *Salmonella* in the samples during the entire storage time under refrigeration for 91 days. Picouet et al. (2012) showed that HPT as an alternative solution for salt reduction in dry-cured meat products in order to obtain healthier food products and good consumer acceptance.

Villamonte et al. (2012) found that HP (350 MPa, 6 min, 20 °C) results in a hardening effect of pork meat batters due to HP-induced changes in myofibrillar protein structure. The structure was destabilized by sodium chloride (1.5–3.0 %) and phosphates (0.25–0.5 %), which counteracted the HP effect on pork batter texture.

**Fig. 4.2** Behavior of (a) *L. monocytogenes* and (b) *Salmonella* in nonpressurized (HP-) and pressure-treated (HP+; 600 MPa/5 min/13 °C) standard (S) and NaCl-free (F) processed smoked dry-cured ham slices during the 112 days of refrigerated storage (From Stollewerk, K., Jofre, A., Comaposada, J., Arnau, J., Garriga, M. 2012a. *Meat Sci.* 90: 472–477. With permission)



Cooking yield and water-holding capacity were improved by the interaction between sodium chloride and phosphates under pressure.

A combination of HPT and nisin in case of cooked ham resulted in a decreased level of *Staphylococcus aureus* by 2.4 log CFU/g after 3-months of storage at 6 °C (Jofre et al. 2008a, b). Furthermore, combined effect of HPT (400 MPa for 10 min) and natural antimicrobials (lactate diacetate or enterocins) controlled the levels of *Listeria monocytogenes* during low storage temperature (Marcos et al. 2008a, b). The combined effect of nisin with HPT on the behavior of *Listeria monocytogenes* inoculated onto the surface of RTE-sliced dry-cured ham indicated that HP as post-processing listericidal treatment is more effective than the use of nisin as an antimicrobial measure (Hereu et al. 2012a). Further, HPT above 450 MPa showed a clear tail-shaped inactivation curve for *Listeria monocytogenes* (Hereu et al. 2012b).

The shelf life of HP-treated (400–600 MPa, 5 min) sliced vacuum-packaged raw ham was extended to 40–74 days when stored at 4 °C. The control sample was spoiled by lactic acid bacteria after 15 days. (Carpi et al. 1999). HP (400 MPa, 15 min) reduced the growth of lactic acid bacteria in vacuum-packaged sliced ham, thereby extending the shelf life from 19 to 85 days (Slongo et al. 2009).

The predominant spoilage organisms of sliced vacuum-packed cooked ham such as *Lactobacillus sakei* and *Lactobacillus curvatus* were found to be highly sensitive to pressure (400 or 600 MPa, 10 min) as they were unable to be detected in HP-treated samples during refrigerated storage. *Weissella viridescens* and *Leuconostoc mesenteroides* survived HPT (600 MPa, 10 min) and were responsible

for the final spoilage (Yanqing et al. 2011). The evaluation of combined effect of HP (200–400 MPa, 10 min) and enterocin LM-2 (256, 2560 AU/g) on the refrigerated shelf life of sliced cooked ham indicated that a combination of 400 MPa and 2560 AU/g enterocin extended the shelf life to more than 90 days and produced a better sensory profile during the storage (Liu et al. 2012). HPT had a discriminant effect on the microbiota of vacuum-packaged sliced cooked pork, which led to the accumulation of different volatile compounds during the refrigerated storage (Clariana et al. 2011a).

### **4.2.2 Restructured Pork**

The combined effect of NaCl, glucono- $\delta$ -lactone (GDL), and  $\kappa$ -carrageenan on the binding properties of restructured pork under hydrostatic pressure indicated that the increase in GDL level leads to a significant decrease in pH and water-holding capacity, but binding strength was found to increase (Hong et al. 2008a). HP (>200 MPa) combined with carrageenan (>1.5 %) has a potential application in cold set binding in restructured pork. It resulted in increased breaking force and tensile strength (Hong et al. 2008b).

HPT (600 MPa) of the transglutaminase restructured dry-cured hams influenced the flavor and the sensory texture attributes, as well as slice. No negative effect on color, flavor, or texture of restructured dry-cured hams was observed by addition of potassium lactate (Fulladosa et al. 2009). The use of K-lactate in combination with HPT (600 MPa) provided an additional reduction in the microbiological counts, increased pink color, brightness, hardness, and saltiness and reduced pastiness and adhesiveness (Fulladosa et al. 2012). HPT (600 MPa, 13 °C, 5 min) increased  $L^*$ , decreased  $a^*$  and  $b^*$  values for restructured ham dried to 20 % weight loss, regardless of salt content and pH.  $L^*$  and  $a^*$  were best preserved in high pH/high salt restructured ham. HPT had no effect on the color of restructured ham dried to 50 % weight loss (Bak et al. 2012).

### **4.2.3 Frozen Ham**

HPT (400 and 600 MPa) of frozen ham resulted in lower visual color intensity as compared to controls, but had no effect on the flavor characteristics of the final product. Higher pressures (600 MPa) showed significantly lower crumbliness and higher fibrousness scores than the control as well as processed at 400 MPa, however, overall sensory quality was not affected (Serra et al. 2007).

HP shift freezing of pork muscle resulted in small and regular crystals. Near the surface there were many fine and regular intracellular ice crystals with well-preserved muscle tissue. From midway to the center, the size of the ice crystals was larger and located extracellularly. Changes in color, reduction in drip loss during

thawing, denaturation of myofibrillar proteins, and reduction in muscle toughness were also observed (Zhu et al. 2004a, b).

HPT (0–600 MPa) resulted in effective microbial inactivation and shelf-life extension of pork carpaccio; product quality decreased due to lower tenderness and poorer appearance. The treatment of carpaccio at  $-35\text{ }^{\circ}\text{C}$  followed by HP resulted in a darker color and a tenderer carpaccio with a higher crumbliness and lower fibrousness and chewiness compared with  $-15\text{ }^{\circ}\text{C}$  (Realini et al. 2011).

#### **4.2.4 Pork Slice and Homogenate**

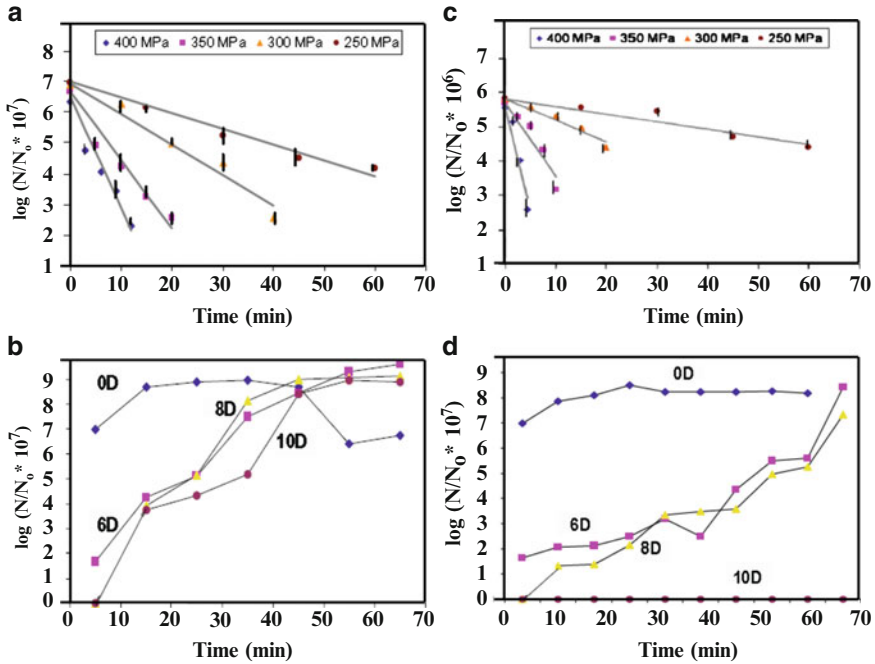
HPT (400 MPa,  $25\text{ }^{\circ}\text{C}$ , 10 min) of pork homogenate (pH 6.7) reduced the populations of *Escherichia coli*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Saccharomyces cerevisiae*, and *Candida utilis* by at least 6 log cycles (Shigehisa et al. 1991). HPT (600 MPa, 3–9 min) was demonstrated to be a useful technique for control of *Listeria monocytogenes* in sliced Parma ham. The treated sample had less red color and intense salty taste (Tanzi et al. 2004). HPT prevented the growth of Enterobacteriaceae, yeasts, and lactic acid bacteria and reduced risks associated with *Listeria monocytogenes* and *Salmonella* in ham leading to enhanced shelf life (Garriga et al. 2004). The increase in pressure and processing time resulted in an increase of  $L^*$  and  $a^*$  values and a decrease in the water-holding capacity of pork. HPT ( $<200\text{ MPa}$ ) of pork for 1 h maintained the quality of cooked pork (Hong et al. 2005). The vitamins riboflavin, thiamin, and thiamin monophosphate were found to be sufficiently stable in HPT of pork (600 MPa,  $25\text{--}100\text{ }^{\circ}\text{C}$ ) (Butz et al. 2007).

HPT (215 MPa, 15 s) reduced the cook and drip loss in pork and the product was more tender than controls (Souza et al. 2011). HPT had no significant effect on the content and fatty acid composition of total lipids and triglycerides in the samples after 7 days of storage at  $4\text{ }^{\circ}\text{C}$ , but treatment at 350 MPa and above led to marked increases in TBARS values and lipolysis of partial phospholipids resulting in an increase of free fatty acid content (He et al. 2012).

### **4.3 Fish**

#### **4.3.1 Inactivation of Microorganisms and Enzymes**

HPT (up to 250 MPa) could not completely inactivate *Listeria monocytogenes* in smoked salmon, but significant lag phases of 17 and 10 days were observed at approx.  $5\text{ }^{\circ}\text{C}$  and  $10\text{ }^{\circ}\text{C}$ , respectively; the treatment had a significant effect on color and texture (Lakshmanan and Dalgaard 2004). At 300 MPa, calpain was almost completely inactivated, but the general protease activity was not affected by HP (Lakshmanan et al. 2005).



**Fig. 4.3** High-pressure survival curves of (a) *E. coli* (O157:H7), (b) *L. monocytogenes* (Scott A) in fish slurry, (c) changes in *E. coli* survivors in HP-treated mackerel during storage at: (b) 20 °C and (d) 12 °C. 0, 6, 8 and 10D are the different processing times. (D-value at 400 MPa is 3 min) (From Ramaswamy, H.S., Zaman, S.U., and Smith, J.P. 2008. *J. Food Eng.* 87: 99–106. With permission)

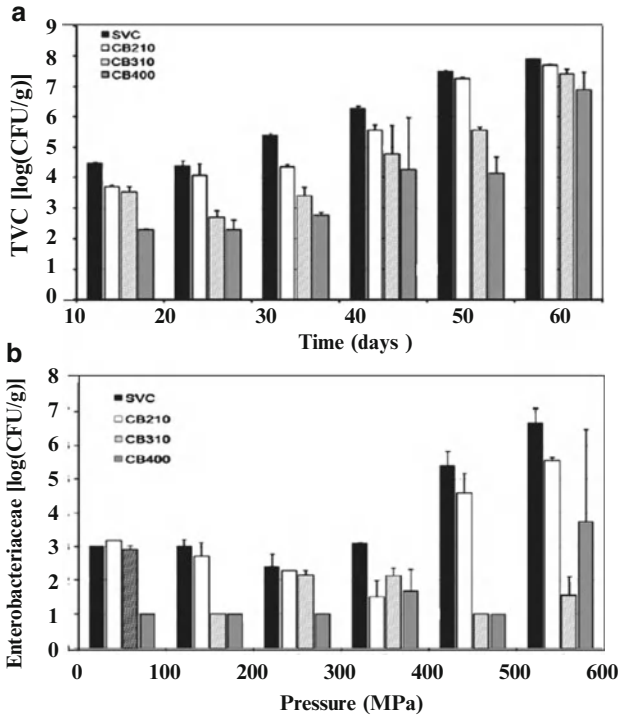
The rate of destruction of *Listeria monocytogenes* was more sensitive to pressure than for *Escherichia coli* in the case of mackerel fish slurry. A 10-D treatment followed by refrigerated storage prevented growth of *Escherichia coli* (Fig. 4.3, Ramaswamy et al. 2008). Most effective conditions for HP inactivation of *Listeria monocytogenes* were 200 MPa, 18 °C, and pH 4.5. Besides, the treatment resulted in an increase of the  $L^*$  value and toughness (Ritz et al. 2008).

HP-treated (220–330 MPa, 3–25 °C, 5 or 10 min) sea bass fillets had higher lightness (Hunter  $L^*$ ) values than controls, whereas TBA values and trimethylamine nitrogen content of sea bass fillets from 10 min HPT (220 MPa, 5 min, 3–25 °C) did not differ significantly from controls (Erkan et al. 2010a).

### 4.3.2 Extended Shelf Life

Fish is a highly perishable commodity and it has a limited shelf life under chill conditions. HPT has been found to effectively extend the shelf life of various fish products. HP-treated sea bass fillets showed increase in lightness and a slight change





**Fig. 4.4** Evolution of the (a) total viable counts (TVC) and (b) Enterobacteriaceae in salmon loins cooked with the sous-vide technique and treated by HP at 210, 310, and 400 MPa (From Picouet, P.A., Cofan C.S., Vilaseca, H., Carbone Ballbe, L., Castells, P. 2011 *Innov Food Sci Emerg* 12: 26–31. With permission)

of hue, decrease of exudation, and water-holding capacity during storage. HPT at >300 MPa led to increased fish hardness after storage than control samples (Cheret et al. 2005). HPT (100 or 300 MPa for 5 min) resulted in decrease in calpain and cathepsin activity, whereas calpastatin was not affected by HPT; but its inhibitory activity decreased during storage (Cheret et al. 2006).

The treatment of Atlantic salmon at 300 MPa and cooking showed higher  $L^*$  and  $b^*$  values but lower  $a^*$  values for dark muscle compared to untreated and treated samples at 150 MPa. The treatment reduced the samples' susceptibility to oxidation. Fatty acid profile of cooked Atlantic salmon dark muscle showed lower amounts of total saturated, n-3 and n-6 polyunsaturated fatty acids and higher amounts of monoenes than HP-treated samples during storage (Yagiz et al. 2009). HPT above 310 MPa extended the shelf life of sous-vide cooked salmon loins by up to 6 days without lipid oxidation and without significant increase in total viable counts and Enterobacteriaceae (Fig. 4.4, Picouet et al. 2011).

The shelf life of untreated and HP-treated (220–330 MPa) sea bream stored at low temperature was 15 days for untreated and 18 days for HP-treated samples

(Erkan and Ueretener 2010). Sea bream fish muscle tissue treated at 200 MPa underwent a decrease in elasticity during storage, whereas the treatment at 300 and 400 MPa preserved elasticity and stiffness during storage. HPT inactivated degrading enzymes acting on proteins that are related to tissue integrity preservation, texture quality, and water-holding capacity (Campus et al. 2010).

HPT (above 200 MPa) or heat treatment (30–70 °C, 30 min) of tilapia meat pastes formed gels. The HP-induced gel was softer and more viscous than that after heat treatment. Subsequent cooking (90 °C, 20 min) increased the hardness, elasticity, and whiteness of the gels both induced by heat and pressure. The appearance of the pressure-induced gels showed glossiness and close to native color of meat pastes, whereas that of the heat-induced gels showed white but no luster (Tan et al. 2010).

Upon HPT (220–330 MPa, 5–10 min, 7–25 °C) of horse mackerel samples there was an increase of  $L^*$  values, whereas  $a^*$  and  $b^*$  values as well as thiobarbituric acid and trimethylamine nitrogen content remained nearly unchanged. The quality of HP-treated horse mackerel appears to have been preserved by HPT (Erkan et al. 2011a). HP-treated (200 MPa) yellowfin tuna chunks vacuum packed in multilayer ethyl vinyl alcohol (EVOH) films and stored at  $2 \pm 1$  °C was acceptable up to 30 days of storage, whereas control samples were acceptable for a period of 20 days (Kamalakanth et al. 2011).

Vazquez et al. (2012) pointed out that HPT (150–450 MPa, up to 5.0 min) prior to freezing resulted in significant inhibition of free fatty acids and tertiary lipid oxidation compound formation for a storage period of 3 months at  $-10$  °C. However, only minor changes in the polyene index and no effect in the content of primary and secondary oxidation compounds were observed.

### 4.3.3 Effect on Freezing and Thawing

High-pressure thawing of blue whiting was quicker and resulted in lower drip volume compared to conventional thawing (Chevalier et al. 1999). Based on the analysis of color, lipid oxidation, and protein stability of fillets, 140 MPa was found to be the optimum pressure level for pressure shift freezing of turbot fillets (Chevalier et al. 2001). High-pressure-thawed dogfish and scallops had better microbial quality, lower thawing time and drip volume than immersion-thawed products (Rouille et al. 2002). High-pressure thawing (200 MPa) resulted in reduced drip loss, improved microbial status, and textural parameters for redfish, haddock, and whiting; but expressible moisture content (water-binding capacity) was higher after conventional thawing (Schubring et al. 2003). Frozen salmon mince followed by HPT and fast pressure release resulted in a 2.5-log-cycle reduction for *Listeria innocua*, whereas pressure-assisted thawing (207 MPa, 10 °C, 23 min) led to a reduction of 1.2 log cycles (Picart et al. 2005). Pressure-shift freezing reduced thawing drip loss compared to air-blast freezing. The freezing process had more influence on the quality parameters than the thawing process (Alizadeh et al. 2007).

#### 4.3.4 Other Marine Products

HPT of vacuum-packed prawn extended its shelf life of 7 days for air-stored samples to 21, 28, and 35 days for vacuum-packaged samples, samples treated at 200, and 400 MPa, respectively. It affected the muscle color only slightly, giving it a whiter appearance (Lopez et al. 2000). High-pressure-induced gels (200–420 MPa) of blue whiting were found to have lower adhesiveness, higher water-holding capacity, and less yellowness than heat-induced gels (Perez and Montero 2000).

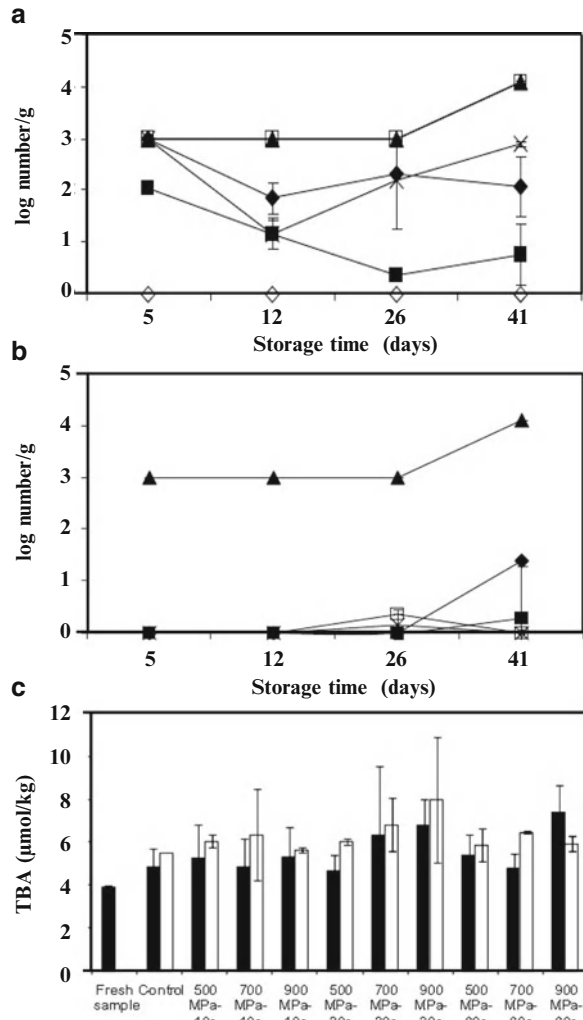
HPT of minced albacore muscle increased pH, reduced lipid oxidation, maintained low microorganisms levels, changed the color of the muscle, and induced the formation of high-molecular-weight polypeptides and improvement in shelf life (Ramirez-Saurez and Morrissey 2006). HPT (100–200 MPa, 4 °C, 15–20 min) of vacuum-packed raw carp fillets (*Cyprinus carpio*) resulted in an increase in thiobarbituric acid-reactive substances and free fatty acids. The color values of the carp fish fillets also increased with pressure and pressurization time (Sequeira et al. 2006).

Cold-smoked dolphin fish pressurized at 300 MPa was quite stable, although sensory attributes declined over storage and fell off sharply after 65 days; HPT did not extend the shelf life, but it was able to diminish bacterial counts during early storage (Gomez et al. 2007a). HPT (300 MPa) reduced the initial microbial population in rainbow trout and mahi-mahi up to 6- and 4-log reduction, respectively (Yagiz et al. 2007). A combination of HPT and edible films enriched with oregano or rosemary extracts yielded the best results in terms of preventing oxidation and inhibiting microbial growth in cold-smoked sardine, thereby increasing its shelf life (Gomez et al. 2007b).

Application of HP (200–300 MPa, 7 °C) was shown to be a tool for obtaining high-quality desalted ‘bacalao’ carpaccio from fish. It resulted in better microbiological quality, thereby increasing the shelf life as well as acquisition of new acceptable sensory attributes by the consumers (Gomez et al. 2009). HPT at 450 MPa for 5 min kept the microbial population of smoked salmon below 6 log<sub>10</sub> CFU/g after 35 days at 5 °C (Medina et al. 2009). HPT (700–900 MPa, 10 s) of cold-smoked salmon resulted in the inactivation of *Listeria innocua* and there was no indication of lipid oxidation and change in redness of the product (Fig. 4.5, Gudbjornsdottir et al. 2010). HPT (220–330 MPa, 3–25 °C, 5–10 min) extended shelf life of red mullet from 12 days to 14 and 15 days for HPT at 220 and 330 MPa, respectively (Erkan et al. 2010b). HPT (150–517 MPa, 20 °C) inactivated *Listeria innocua* of minced trout (*Oncorhynchus mykiss*). The treatment at 414 MPa for 5 min achieved greater than a 4-log reduction for the *Listeria* strains. Addition of salt increased the effectiveness of pressure treatment (Akgul et al. 2010).

HPT (220–330 MPa, 3–25 °C) significantly changed the sensory, chemical, and microbiological properties of cold-smoked salmon, and in combination with adequate chilled storage improved the shelf life and safety. Sensory and microbiological results indicated that HP-treated cold-smoked salmon samples were acceptable up to 8 weeks compared to control samples only up to 6 weeks (Erkan et al. 2011b). HPT (200 MPa, 3 min) in the case of vacuum-packed fresh herring and haddock

**Fig. 4.5** Growth of *Listeria innocua* (MPN) during storage of cold-smoked salmon at 5.5 °C. Cold-smoked salmon had been treated with (a) 500 MPa and (b) 900 MPa for 10 (□-), 20 (-x-), 30 (-◆-) and 60 (-■-)s. *Listeria* concentration before pressure was  $4.5 \times 10^3$  CFU/g; (c) Changes in TBARS value as an indicator for rancidness during storage at 5.5 °C for 18 (black column) and 41 (blank column) days after HPT (From Gudbjornsdottir, B., Jonsson, A., Hafsteinsson, H., Heinz, V. 2010. *Lebensm. Wiss. Technol.* 43: 366–374. With permission)



extended the microbiological shelf life of fish from 4 to 13 days (Karim et al. 2011). HP (100–300 MPa, 15–30 min) can be used as a tool for quickly obtaining fish skin gelatin hydrolysates with antioxidant capacity. Hydrolysis performed by HPT increased the degree of hydrolysis with all the enzymes alcalase, collagenase, trypsin, and pepsin, whereas the radical scavenging capacity of the hydrolysates was enhanced when alcalase or collagenase were used for hydrolysis (Aleman et al. 2011). HPT (400 MPa, 10 min or 500 MPa, 5 min) of smoked cod reduced microbial counts and delayed microbial growth during refrigerated storage. No difference in lipid oxidation was found after pressurization and during storage (5 °C, 60 days) (Montiel et al. 2012a). Further, Montiel et al. (2012b) demonstrated that HPT (450 MPa, 10 min) combined with the lactoperoxidase system in case of

cold-smoked salmon can be used as a hurdle technology approach against *Listeria monocytogenes*, increasing the safety and the shelf life during refrigerated storage.

Vacuum-packed prawns in EVOH multilayer pouches subjected to HPT (100–600 MPa) and stored in chilled ( $2 \pm 1$  °C) condition resulted in an increase of pH and TBA values after HPT and significantly increased on storage. TMA and TVB-N values were reduced after HPT, which were gradually increased during storage. Hardness, whiteness, and yellowness increased with increasing pressure, and redness was found to decrease (Bindu et al. 2012).

## 4.4 Poultry

HPT (400 MPa, 50 °C, 10–30 min) of fatty goose or duck liver resulted in reduction in microbial load while maintaining its texture, flavor, and yield. Further, there was no melting or separation of lipids as a result of pressure treatment, in contrast to the 15 % lipid loss due to thermal pasteurization (El Moueffak et al. 1996). A combination of HPT (350–550 MPa) and temperature (55–65 °C) was used to give a product of similar microbiological quality to that obtained by heat treatment. The pressure treatment (550 MPa, 20 min, 55 °C) resulted in a 7-log-cycle reduction of the total aerobic mesophilic flora and *Enterococcus faecalis* (El Moueffak et al. 2001). Pathogenic avian influenza A virus in cell culture medium and chicken meat can be inactivated by HPT (500 MPa, 15 °C, 15 s). While raw chicken meat is oxidatively stable, HPT at 600 MPa and above may induce lipid oxidation resulting in off-flavors during subsequent cooking (Isbarn et al. 2007).

An inactivation strategy for *Clostridium perfringens* spores in poultry meat consisted of a primary heat treatment (80 °C, 10 min) to pasteurize and denature the meat proteins and to activate the spores for germination, cooling of the product to 55 °C for spore germination, and finally inactivation of germinated spores by pressure-assisted thermal processing (586 MPa at 73 °C, 10 min) (Akhtar et al. 2009). *Weissella viridescens* was found to be the dominant microorganism in the pressure-treated vacuum-packaged cooked poultry meat. Studies on a typical *W. viridescens* isolate showed it to be relatively pressure-resistant in poultry meat, with <1 log reduction in numbers after a treatment of 2 min at 600 MPa (Patterson et al. 2010). HPT (600 MPa, 2 min, 20 °C) alone was not sufficient to eliminate all of the *Listeria* inoculated into cooked chicken. Numbers of survivors were initially below the level of detection but increased during storage at 8 °C and reached  $> 10^8$  CFU/g in 21 days. The addition of 2 % w/w sodium lactate in combination with pressure treatment was found to be most effective at inhibiting the growth of *Listeria monocytogenes* and numbers remained below the limit of detection throughout the 105-day storage period (Patterson et al. 2011).

Color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) were significantly higher for pressurized chicken breast fillets than for controls. When single-cycle treatments were applied, maximum values of texture parameters were generally found for fillets pressurized for 10 or 15 min and decreased for fillets submitted to longer cycles. In the case of

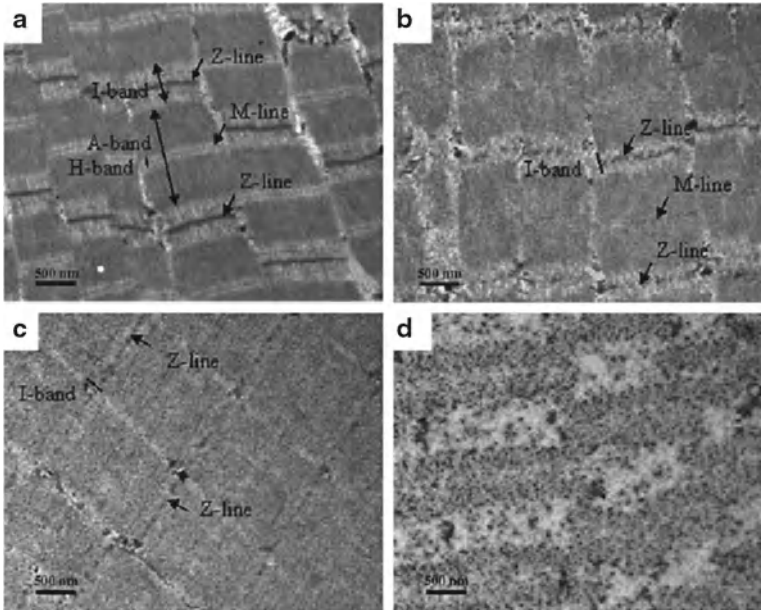
multiple-cycle treatments, texture parameters initially increased with the number of 1-min cycles, but fillets submitted to the most severe treatments showed lower values (Olmo et al. 2010).

Application of HP (200 and 400 MPa) with cooking at 70 °C resulted in formation of less compact aggregated microstructures having better binding properties and were less hard than controls (Fernandez et al. 1998). HP application (2000–400 MPa, 30 min) resulted in increased water- and fat-binding properties of chicken and pork batters even at low ionic strengths (Jimenez et al. 1998).

HPT (up to 500 MPa) of chicken breast muscle did not result in rancidity during chilled storage and product was similar to controls. Pressure treatment at 600 and 700 MPa resulted in less oxidation, but at 800 MPa lipid oxidation increased to the same extent as in heat treatment (Orlien et al. 2000). HPT (400 or 600 MPa) resulted in an increase in secondary lipid oxidation products (hexanal, octanal, and nonanal) in the cooked breast chicken compared to 200 MPa treatment and controls (Wiggers et al. 2004). HPT of chicken breast and thigh resulted in the formation of free radicals the extent of which increased with an increase in pressure (Bragagnolo et al. 2006). Addition of 0.1 % dried rosemary to minced chicken thighs or breasts prior to HPT inhibit lipid oxidation during subsequent cooking (Bragagnolo et al. 2007). HPT (600–800 MPa, 50–70 °C) induced increased rates of lipid oxidation in chicken muscle. Addition of 1 % of EDTA (ethylenediaminetetraacetic acid) disodium salt inhibited the increased rates of lipid oxidation due to release of transition metal ions from insoluble complexes, which catalyzes lipid oxidation in pressure-treated muscle foods (Ma et al. 2007). Addition of sage protected minced chicken breast processed with HP (up to 800 MPa, 10 min) against lipid oxidation during subsequent chilled storage for 2 weeks; addition of garlic showed a prooxidative effect partly counteracted by simultaneous addition of sage (Mariutti et al. 2008).

HPT was a suitable alternative to heat treatment for inactivating *Salmonella enteritidis* and mesophiles in poultry sausage manufacture (Yuste et al. 2000). The highest decrease in mesophiles (5.3 log CFU/g) and psychrotrophs (>7.5 log CFU/g) counts were observed in mechanically recovered poultry meat after exposure of the meat to 450 MPa pressure in the presence of nisin (200 ppm) (Yuste et al. 2001, 2002). The packaging of HP-treated (550 MPa, 55 °C, 20 min) fatty duck liver in ethylene and vinyl alcohol copolymer (EVOH) films resulted in a product with extended shelf life of 90 days at 4 °C coupled with a significant fat loss. HPT decreased the total aerobic mesophilic counts and completely inactivated coliform bacteria (Cruz et al. 2003).

HPT (150 MPa) was demonstrated to be a technique for the salting of turkey breast meat. Water and sodium chloride diffusion into the sample was found to be maximal at 150 MPa. Examination of the microstructure of HP-treated sample indicated swelling of myofibrils, disappearance of the M line, reduced difference in the density of the A band and I band, and breaking of segments of the Z line (Fig. 4.6, Villacis et al. 2008). Use of curdlan instead of fat and the application of HPT (>300 MPa) achieved low-fat (<6 % fat) and low-salt (1 % salt) duck muscle gel products with good properties and high yields (Chen et al. 2010). The exposure of chicken mince products containing modified starch to a pressure of 300 MPa for



**Fig. 4.6** Microstructure of control and pressure-treated turkey breast samples: (a) control, (b) salting at 0.1 MPa for 2 h, (c) salting at 150 MPa for 15 min; (d) salting at 300 MPa for 15 min, 50 g/l NaCl solution was used for salting the meat (From Villacis, M.F., Rastogi, N. K., and Balasubramaniam, V. M. 2008. *Lebensm. Wiss. Technol.* 41: 836–844. With permission)

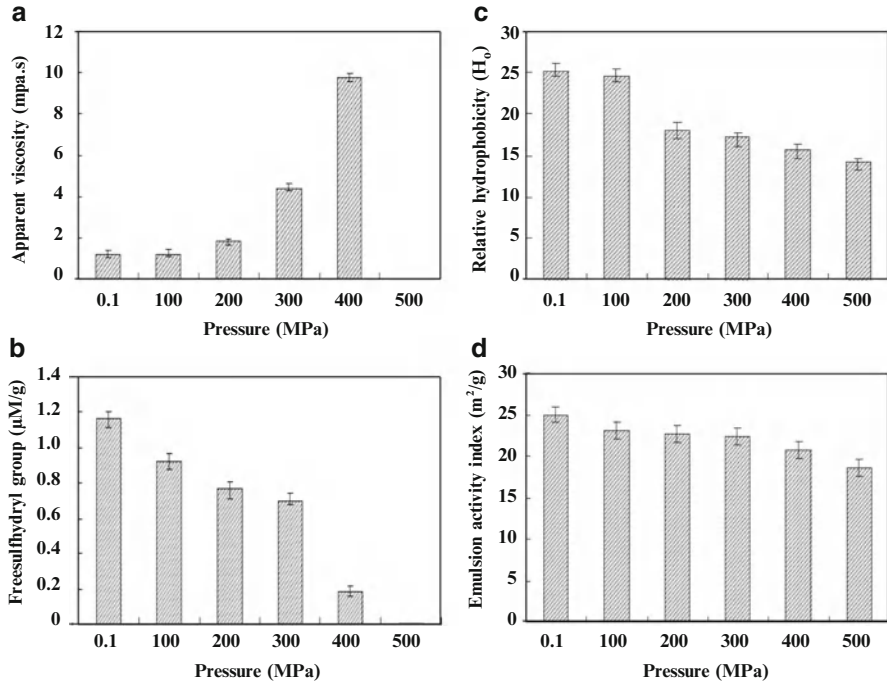
20 min resulted in optimal-quality chicken products in terms of water-holding capacity, gel strength, and whiteness (Min and Ming 2010).

The patties made of minced chicken breast and thigh packed in antioxidant-active packaging, subjected to HPT (800 MPa, 10 min, 5 °C) and subsequently stored for 25 days at 5 °C resulted in higher lipid oxidation in the surface part than inner part. The active packaging was able to delay lipid oxidation up to 25 days (Bolumar et al. 2011). HPT (400/600 MPa, 40 °C) of chicken breast meat with addition of  $\beta$ -glucan and NaCl formed gel in the absence of sodium tripolyphosphate similar to that produced with 2.5 % NaCl. HPT and addition of  $\beta$ -glucan can help to partially replace salt addition in chicken products produced by HPT (Omana et al. 2011a, b).

## 4.5 Eggs

HPT (300 MPa, 200 s) did not lead to deterioration of the functional properties of eggs and could be used as a preservation technique with addition of antimicrobial agents in which the sensory properties and nutrient contents of liquid whole egg were maintained (Schenkova et al. 2009). HPT (100–500 MPa) resulted in protein





**Fig. 4.7** (a) Apparent viscosity, (b) free sulfhydryl group, (c) relative hydrophobicity, (d) emulsion activity index values of untreated (0.1 MPa) and HP-treated (100–500 MPa) egg yolk (From Yan, W., Qiao, L., Gu, X., Li, J., Xu, R., Wang, M., Reuhs, B., Yang, Y. 2010. *Euro. Food Res. Technol.* 231: 371–377. With permission)

aggregation, as evidenced by gradual decrease in protein solubility and significant increase in viscosity. The treatment induced a gradual decrease in hydrophobicity and free sulfhydryl content, possibly due to protein unfolding and subsequent aggregation/reassociation of unfolded proteins. Emulsifying activity index was slightly decreased between 100 and 300 MPa, and at pressures above 400 MPa it was significantly decreased relative to the untreated egg yolk (Fig. 4.7, Yan et al. 2010).

Lai et al. (2010a) studied the effect of HPTs (300–500 MPa, 25 °C, 10 min) on changes in the physicochemical properties of egg white and yolk proteins of duck eggs. HPT at 500 MPa showed that egg white proteins underwent slight but significant unfolding and aggregation, whereas HPT below 500 MPa induced insignificant changes in the physicochemical properties. Pressure treatments at 400 and 500 MPa significantly reduced the solubility. Monfort et al. (2012) indicated that HPT (300 MPa, 3 min) followed by heat (52 °C, 3.5 min or 55 °C, 2 min) to liquid whole egg in presence of 2 % triethyl citrate offered liquid whole egg with the same microbial safety level as the industrially treated product at 71 °C for 1.5 min, but with quality properties similar to fresh liquid whole egg. Hoppe et al. (2012) demonstrated that HPT (800 MPa) of egg white resulted in greater



susceptibility to pepsin hydrolysis than thermal treatment at 95 °C. The analysis of the low-molecular-weight fraction (3.0 kDa) derived from pepsin-digested pressure-treated egg white contained numerous peptides having bioactive and/or immunological properties.

## 4.6 Sausages

HPT (600 MPa, 10–40 °C, 20 min) of sausages resulted in decrease in counts of *Listeria monocytogenes* by 4–5 log cycles. The redness was found to decrease, whereas lightness and hardness of the sausage batter increased. The treatment of sausage batter at 600 MPa modified the IgE immunoreactivity of proteins (Hajos et al. 2004). HPT (300 MPa, 10 min, 17 °C) was used as an additional hurdle to the ripening process for causing a greater decrease in the *Salmonella* population (3°MPN/g) in ripened low-acid fermented sausages (Fuet and Chorizo). Lower values of *Listeria monocytogenes* counts were obtained in untreated than in pressurized sausages due to delay in pH drop caused by HP inactivation of endogenous lactic acid bacteria. A discoloration (increase in  $L^*$  values) of the sausages was observed after HPT (Marcos et al. 2005). The addition of enterocins A and B to raw sausages and HPT (400 MPa) at the end of the ripening process produced fermented sausages with an immediate reduction in the counts of *Salmonella*, while *Listeria monocytogenes* and *Staphylococcus aureus* were not reduced. Storage at room temperature or at 7 °C resulted in decrease in the counts of *Salmonella* and *Listeria monocytogenes*, which progressively decreased to less than 1 CFU/g (Jofre et al. 2009b). The combined HPT (350 MPa, 20 min) and addition of *Lactobacillus casei* cell extract (CE) to inhibit growth of *Escherichia coli* O157 in broth and sausage resulted in 1.1–1.2 log reduction, whereas combined HP with cell extract (32 CEAU/ml) led to viability loss of 4.3–4.6 logs (Chung and Yousef 2010).

Application of HPT (400 or 800 MPa) did not significantly increase cholesterol oxidation product concentration (related to incidence of cardiovascular disease) in cooked sausages immediately after treatment or storage up to 3 weeks at 2 °C (Muench et al. 2005).

The amount of released and expressible water significantly decreased with increasing pressure (300–700 MPa), temperature (40°C and 60 °C) and holding time (40 and 60 min) for ostrich meat sausages (yor or Thai sausage). Besides, gel strength and equilibrium stress were increased with increasing severity of treatment. Pressures of 700 MPa yielded gel networks involving completely denatured protein with ability to retain water (Supavitpatana and Apichartsrangkoon 2007). HPT (200–600 MPa) of ostrich-meat changed the viscoelastic properties, which resulted in structural changes due to hydrophobic interactions and disulphide bonding. Pressure treated products received higher sensory scores than conventionally steamed products (Chatton and Apichartsrangkoon 2009).

Raw dry sausages subjected to HPT (600 MPa, 10 min, 20 °C) after 28 days of ripening resulted in reduction in total count (by a factor of  $10^3$ ), inactivation of lactic

acid bacteria, increased firmness, paler color, increased fat oxidation, and reduced fat hydrolysis. Overall sensory quality of HP-processed sausages was same as the control samples (Dederer and Mueller 2008). A combination of HPT and heat treatment to canned Bruehwurst sausages resulted in inactivation of all spores; the canned products are stable under tropical conditions with poor sensory quality. The pressure-induced germination of spores (at 300 MPa) followed by pasteurization resulted in spore destruction while maintaining high sensory quality (Mueller and Dederer 2008).

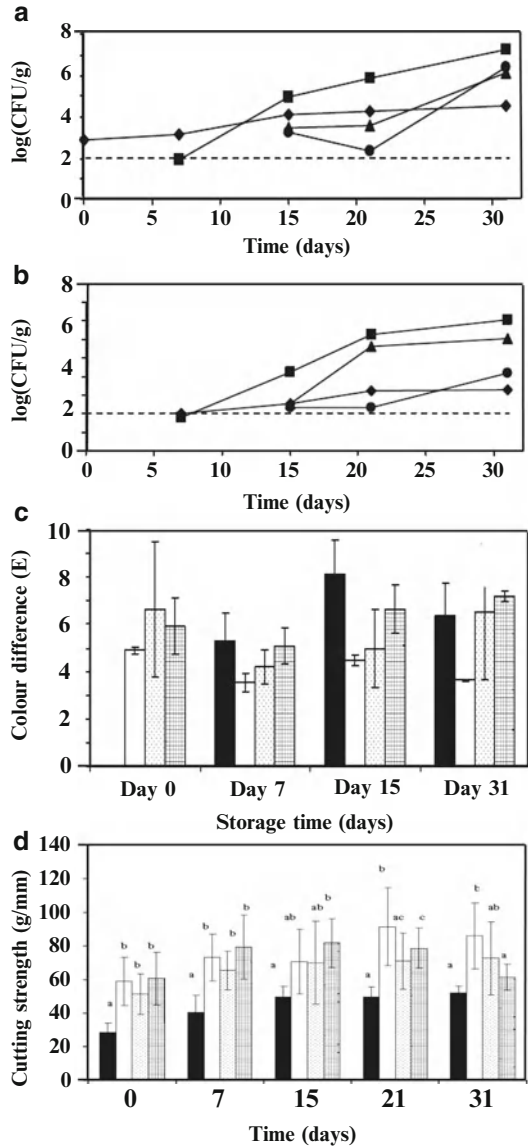
The cooking of raw meat batters packed into casings with 1 % NaCl concentration treated with HPT (400 MPa, 2 min, 10 °C) resulted in 9.3 % cooking loss, whereas the loss in unpressurized samples was 24.9 % cooking loss. The moisture retention, hardness, and gumminess of pressure-treated samples were higher compared to untreated samples (Sikes et al. 2009). HPT (500 and 600 MPa) and addition of carrot dietary fiber markedly improved emulsion strength, which resulted in firm sausages, besides improvement in sensorial attributes like homogeneity, creaminess, fattiness, and firmness (Grossi et al. 2011). The effect of HPT (400 MPa, 10 min, 12 °C) on the volatile compounds of low-acid-fermented sausage “espetec” and sliced cooked pork shoulder indicated that the effect of pressurization on the volatile fraction of “espetec” was better categorized by dynamic headspace extraction, whereas solid-phase microextraction was more appropriate for cooked pork shoulder (Canedo et al. 2012).

## 4.7 Oysters

HPT is being increasingly employed for commercial processing of oysters. It is continuously gaining acceptance among oyster processors due to its ability to shuck oysters while keeping the fresh-like characteristics of oysters (Rastogi 2010).

HPT (100–800 MPa, 10 min, 20 °C) of oysters resulted in inactivation of pathogens, increase in moisture content and pH, decrease in protein and ash contents, detachment of oyster muscles from shells (shucking), and maintenance of good shape. The pressure-treated tissues were more voluminous and juicy compared to untreated oysters. The total color difference showed that there were significant differences in color between samples treated by heat or pressure (Cruz et al. 2004, 2007). HPT (260–800 MPa) significantly changed the microflora of oysters and apparently has good potential for inactivation of *Vibrio* spp., which can improve the microbiological shelf life and safety of oysters during chilled storage for 14 days (Cruz et al. 2008a). It resulted in no significant changes in the fatty acid profile compared to untreated oysters, but the concentrations of volatile compounds were altered (Cruz et al. 2008b). Besides, it delayed the microbial growth in chilled stored oysters, which can affect quality attributes. During the storage of sample, the color turned yellow and hardness increased (Fig. 4.8, Cruz et al. 2008c). Fletcher et al. (2008) indicated that shucking of New Zealand Greenshell mussels (*Perna canaliculus*) by HPT (400 MPa) has potential benefits in product quality, increased yield, and inactivation of *Listeria monocytogenes*.

**Fig. 4.8** Oysters: changes in (a) total viable counts, (b) anaerobic plate counts during storage on ice under refrigeration (2 °C) of untreated oysters (◆) or HP-treated oysters at 260 (■), 400 (▲), or 600 (●) MPa. The dotted line shows the limit of detection. (c) Changes in the total color differences (d) cutting strength during chilled storage (2 °C) of untreated oysters (■) or HP-treated oysters at 260 (□), 400 (▨), or 600 (▩) MPa. (From Cruz, M.R., Kerry, J.P., Kelly, A.L. 2008c. *Food Cont.* 19: 1139–1147. With permission)

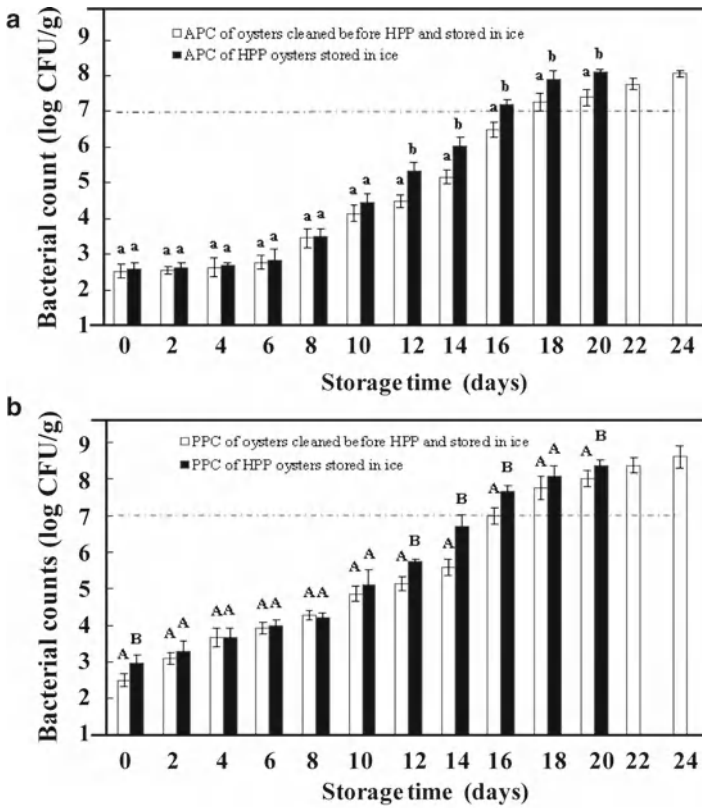


Higher baroresistance of bacteria in oysters than in buffer indicated that studies of HP-induced bacterial inactivation in buffer systems may not predict inactivation of microorganisms in foods (Smiddy et al. 2005). Kural et al. (2008) achieved a 5-log reduction of *Vibrio parahaemolyticus* in live oysters, for a pressure treatment  $\geq 350$  MPa for 2 min at 1 and 35 °C and  $\geq 300$  MPa for 2 min at 40 °C. Kural and

Chen (2008) indicated that HPT ( $\geq 250$  MPa,  $-2$  or  $1$  °C,  $\leq 4$  min) resulted in a 5-log-cycle reduction of *Vibrio vulnificus*, which is frequently associated with oysters. Six-log-cycle reduction and virtual inactivation of hepatitis A virus (HAV) was achieved by HP (350–400 MPa) (Calci et al. 2005). Li et al. (2009) demonstrated that HPT (200–400 MPa, 5 min,  $0$  °C) reduced murine norovirus-1 contamination of oysters to undetectable levels. Kingsley et al. (2009) showed commercial HPP applied to whole in-shell oysters to be capable of inactivating HAV. HPT (400 MPa) of whole in-shell oysters and shucked oysters led to average log reduction values of 2.56 and 2.96 for inactivation of HAV, respectively, i.e., no significant difference between inactivation for whole in-shell oysters and shucked oysters. Terio et al. (2010) evaluated the potential of HPT (300–400 MPa,  $18$ – $22$  °C) to inactivate HAV within blue mussels (*Mytilus edulis*) and Mediterranean mussels (*Mytilus galloprovincialis*). For blue mussels,  $\log_{10}$  PFU reductions of HAV averaged 2.1 and 3.6 at HPT of 350 and 400 MPa, while for Mediterranean mussels reductions of 1.7 and 2.9  $\log_{10}$  PFU were observed for equivalent treatments. Ma and Su (2011) evaluated the effect of HPT on *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*). A treatment at 293 MPa for 120 s at groundwater temperature ( $8 \pm 1$  °C) achieved greater than 3.52-log reductions of *V. parahaemolyticus*. Oysters processed at 293 MPa for 120 s had a shelf life of 6–8 days when stored at  $5$  °C or 16–18 days when stored in ice. Washing oysters before HPT increased the shelf life of oysters stored in ice to 17 days (Fig. 4.9).

Hsu et al. (2010) showed HPT (250 and 300 MPa) to be a good method for oyster shucking. The pH of HP-treated oysters increased slightly from 6.50 to 6.82, and the moisture content of the HP-treated oysters was higher than those of controls. The brightness, yellowness, and cutting strength of HP-treated oysters with further cooking did not change, while the redness decreased compared to the controls. Lai et al. (2010b) indicated that 300 MPa for 2 min is the optimum HPT that resulted in oysters most acceptable for oyster omelets during storage at  $4$  °C, and this treatment may extend the shelf life of these oysters to 21 days.

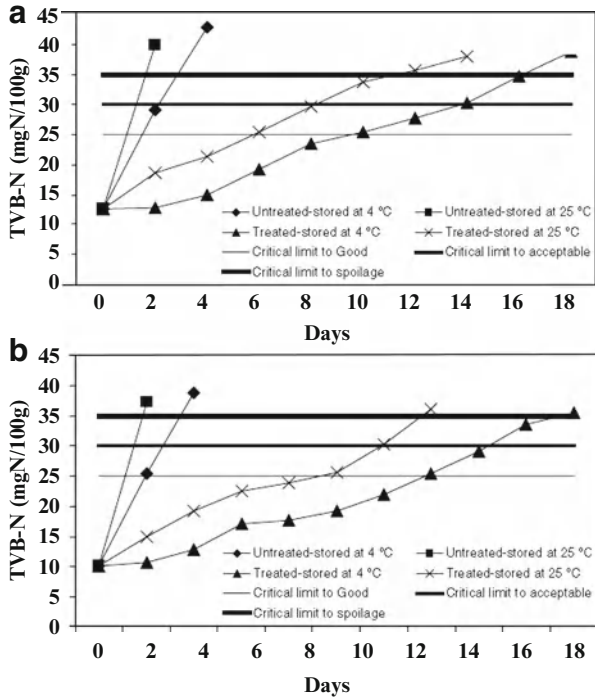
Prapaiwong et al. (2009) showed that numbers of total aerobic bacterial counts (TABC) in HP-treated oysters were significantly lower than in untreated oysters. However, TABC reached  $10^8$  CFU/g at 14 days of storage, which indicated that some bacteria survived the treatment and were able to proliferate during refrigeration conditions. Analysis of the bacterial flora by 16S rDNA sequencing, revealed six different classes of bacterial communities. Labarca et al. (2012) evaluated the effect of HP (500–550 MPa for 3–8 min) on quality changes (microstructure, color, texture, and biochemical) of red abalone (*Haliotis rufescens*) during storage at  $4$  °C. The HP-treated abalones were found to have higher pH, moisture, and ash content than untreated abalones, whereas protein and fat contents of treated abalones were lower. Total volatile basic nitrogen and trimethylamine contents were found to increase in HP-treated samples, but the maximum concentration did not exceed the allowed limit in 60 days of storage.



**Fig. 4.9** Oysters: changes of (a) aerobic plate counts (APC) and (b) psychrotrophic plate counts (PPC) in HP-treated oysters (293 MPa at  $8 \pm 1$  °C for 120 s) stored in ice. Initial levels of APC and PPCs in oysters before HPT were 3.19 and 3.99 log CFU/g, respectively. The dotted line indicates spoilage of products (From: Ma, L., Su, Y.C. 2011. *Intl. J. Food Microbiol.* 144: 469–474. With permission)

### 4.8 Shrimp, Clams, and Squid

Buyukcan et al. (2009) demonstrated that HPT (200–250 MPa, 25–50 °C, 10–20 min) enhances the shelf life of clams (*Venus gallina*) and shrimp (*Parapenaeus longirostris*) samples from 4 days to 16 days and 18 days at 4 °C, respectively, whereas it was 12 days at room temperature (Fig. 4.10). Narwankar et al. (2011) indicated that at least 480 MPa pressure was needed to achieve 1-log reduction in quahog (hard clams, *Mercenaria mercenaria*). The consumers showed equal preference for processed and raw quahogs. Arcangeli et al. (2012) showed that at least 500 MPa for 1 min was found to be effective in inactivating the murine norovirus in contaminated Manila clams. Gou et al. (2010) demonstrated that HPT (300 MPa, 20 min) inhibited trimethylamine-*N*-oxide demethylase (TMAOase) activity in squid



**Fig. 4.10** Total volatile basic nitrogen values of HP-treated and untreated (a) shrimps and (b) clams at 4 °C and 25 °C storage (From Buyukcan, M., Bozoglu, F., Alpas, H. 2009. *Intl. J. Food Sci. Technol.* 44: 1495–1502. With permission)

during 12 days of refrigerated storage, which effectively reduced the production of dimethylamine. Therefore, HPT could be a promising alternative to retard the quality deterioration of squid. Gou et al. (2012) demonstrated that HPT (500 MPa, 10 min) of semidried squid effectively retarded the growth of *Morganella morganii* and *Klebsiella pneumonia* contributing to the formation of biogenic amines and also inhibited the development of off-flavors such as dimethylamine and trimethylamine during refrigerated storage.

## Chapter 5

# Conclusion

High-pressure processing can reduce or eliminate microorganisms of concern in food without deteriorating product quality or compromising safety, which can justify the substantial capital investment for adopting this technology on an industrial scale. Quantification of the impact of high pressure on safety is critical for consumer acceptance as well as for its use in the food processing industry. Heat-sensitive products are particularly suited for treatment by high-pressure processing.

The growing availability of high-pressure-processed products such as fruits juices, guacamole, meat products, oysters, and dairy products on the international market indicates an increasing acceptance and potential for the future. High-pressure processing may find its niche in particular applications rather than replacing traditional technologies. This technology will benefit consumers in terms of increased food safety, extended shelf life, and extraordinary quality besides providing diversified value-added nutritious food products at reasonable cost. The initial installation and startup of high-pressure facilities does require a major capital investment, this however is counterbalanced by lower operating costs and higher product quality. As the demand for high-pressure processing equipment is steadily growing this indicates that equipment costs will soon decrease. Research scientists, food companies, and regulatory authorities (such as the U.S. FDA) are engaged worldwide to ensure that the novel high-pressure-processed products comply with the particular food safety requirements.

Consumer surveys have indicated growing acceptance of high-pressure-processed foods and willingness to pay slightly higher prices for the enhanced safety and quality of the products.

Nutraceuticals, functional food ingredients and related compounds can also benefit from high-pressure processing where functionality before has been compromised by extensive heat treatment. Fish and shellfish industries are adopting this new technology because it accomplishes the near elimination of pathogenic or spoilage microorganisms at or near room temperature. Raw/fresh oysters can be treated by these nonthermal means to reduce bacterial loads without causing significant changes in appearance, flavor, texture, and nutritional qualities. Salads are ideally suited for

high-pressure treatment. Juice vitamin C content upon high-pressure treatment has been shown to be similar to that of fresh juice after 12 weeks of refrigerated storage. Heat-sensitive products such as pasteurized liquid egg, seafood, and other minimally processed foods also benefit from the advantages regarding food safety resulting from high-pressure treatment.

While high pressure has been shown to effectively inactivate various kinds of microorganisms, the issue of foodborne viruses still needs to be further addressed. Through ongoing research, development, and education and by demonstrating this new technology to entrepreneurs regarding its potential for improving product quality and development one can surely say that high-pressure food processing is here to improve the quality of foods on the market where conventional methods fail to yield satisfying results.



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