

Review

Effect of High Pressure Processing on the Microbial Inactivation in Fruit Preparations and Other Vegetable Based Beverages

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Abstract: The purpose of this study is to review the effects of High Hydrostatic Pressure Processing (HPP) on the safety of different fruit derivatives (juices, nectars, jams, purees, pastes . . .), considering the types established in the European legislation and some other vegetable-based beverages (mainly juices and smoothies). The main inactivation processes and mechanisms on microorganisms are reviewed. Studies have revealed that HPP treatment is capable of destroying most microorganisms, depending on the application conditions (amplitude of the pressure, duration time, temperature, and the mode of application), the properties of the fresh and processed fruit/vegetables (pH, nutrient composition, water activity, maturity stage), and the type of microorganisms or viruses.

Keywords: High Pressure Processing (HPP); fruit preparations; vegetable based beverages; food safety; microbial inactivation

1. Introduction

One main concern in the food process industry is the survival and growth of pathogens, causing bacterial spoilage and human infections, and affecting the health and safety of consumers. Nowadays, new processing technologies are needed to deliver products in compliance with requirements, while maintaining other main quality attributes such as nutritive and sensory properties. One of these treatments that was applied in the food industry at the end of the 80s, is based on the application of high pressure into jams, fruit jellies, sauces, and fruit juices. This technique can inactivate microorganisms and enzymes, prolonging food shelf life with minimal effects on nutritive and organoleptic quality. Therefore, HPP treatment seems to be a better alternative to other traditional techniques such as thermal pasteurization, used to preserve food products.

Minimally processed fruits and vegetables is one of the major growing sectors in the food industry [1]. Moreover, beverages, concentrated juices, and purees are vital food products, due to the massive demand of the global market [2]. The importance of minimally or non-thermally processed foods with an increased shelf life and better nutritional properties is increasing [3]. Nowadays, fruits and vegetables are included in a food sector in which pressurizing techniques are mostly used, reaching a 25% share, of the market for pressurized foods. See data on Figure 1 that summarizes articles published in the last 19 years. Processing on these types of products will help to better understand the application of pressurization technologies.

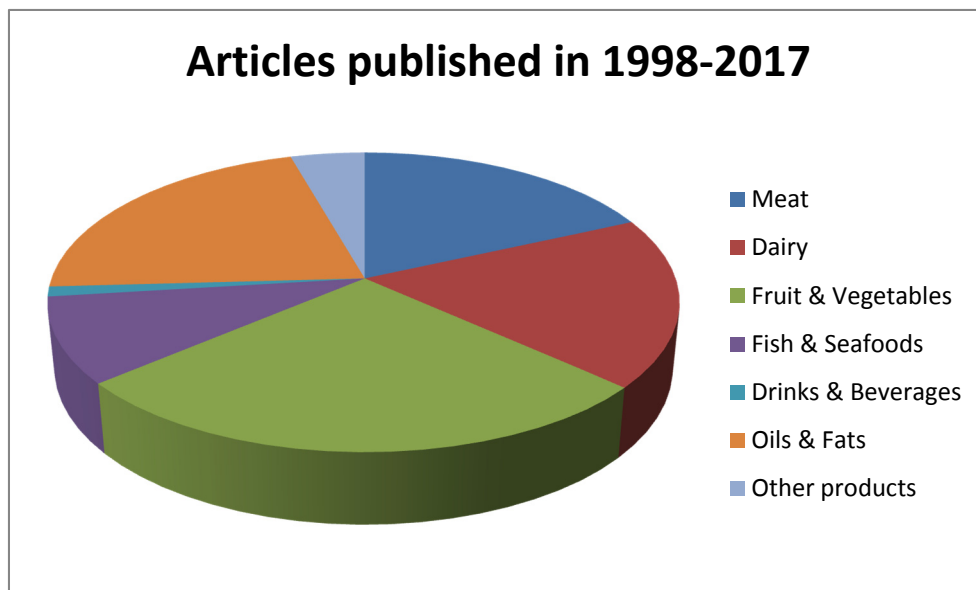


Figure 1. Food processed by High Hydrostatic Pressure Processing (HPP) technologies (period 1998–2016) divided by sectors.

In order to protect consumers' health, food safety is an important parameter for placing these products on the food market. For instance, fruit juices can be contaminated with pathogenic microorganisms that can grow and survive, causing health problems for consumers [4,5]. Bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria* spp. are the pathogens most frequently linked to fruit and vegetable produce-related outbreaks, being a public health concern [6,7].

Therefore, in this study the revision of pressurization effects on the safety of processed vegetable and fruit preparations (juices, purees, jams, smoothies...) at pressures from 100–700 MPa, temperatures ranging from minus 10 °C–90 °C, and processing times of 1–20 min, will be visited.

2. High Pressure Processing in the Food Industry

High Pressure Processing, also known as pascalization, is a non-thermal pasteurization consisting of treatments above 100 MPa (in the food industry, this pressure range usually varies between 100–700 MPa). Pressure generation occurs through the mechanical pressure exerted on the fluid contained in the machine and consequently transmitted to the product. This pressure is applied on the liquid, usually water. This liquid is transmitted to a vessel where the product is already contained within its packaging, and this pressure is held for a given period of time. The pressure is transmitted uniformly and instantaneously throughout the food, which allows very homogeneous products to be obtained [8].

The basic principles that determine the behavior of foods under pressure, described by some authors [9] are:

Le Chatelier's principle: any reaction, conformational change, phase transition, accompanied by a decrease in volume is enhanced by pressure [10].

Principle of microscopic ordering: at constant temperature, an increase in pressure increases the degrees of ordering of molecules of a given substance. Therefore, the temperature is increased as long as the pressure applied is increasing (between 2–3 °C per each 100 MPa).

Isostatic principle: the products are compressed by uniform pressure from every direction and then returned to their original shape when the pressure is released [11]. So, the products are compressed independently of the product size and geometry because transmission of pressure to the core is not mass/time dependent; thus the process is minimized [12].

The first studies confirming the efficiency of the treatments of foods by high pressures dates back to the end of the 19th century. Hite tried to prevent milk from spoiling, and his work showed that microorganisms can be inactivated by processing milk with high pressure [12].

However, this technology took almost 80 years to be adapted for foods. The first food products treated by this process and commercialized appeared in 1990, in Japan. These initial food products were prepared fruits: juices, jellies, and jams. The Japanese then diversified pressurized products (meats, fishes, rice puddings, ham of ox, sake, etc.), as well as the range of the machines used for food treatment. Afterwards, the process expanded beyond the Japanese borders, and other countries took up this new processing technology. Nowadays, pressurization is considered an emerging processing technology used to obtain a great variety of fruit-based products, and reduces energy cost in the food industry [13].

The numerous advantages of this process have been praised and mentioned: a degradation of the bacterial flora with minimal heat treatment, no modification of vitamins, a low modification of color and taste, and the inactivation of enzymes. All of these effects allow the extension of food shelf life while preserving their nutritional and organoleptic properties. Moreover, this treatment is seen as an alternative to thermal pasteurization [14], especially in preparations such as fruit juices [15].

The HP operation can be divided into two categories: (1) High pressure pasteurization at 300 to 600 MPa for 1–15 min and at the initial product temperature of 5–25 °C, in order to inactivate vegetative pathogens; and (2) High pressure sterilization, or HPHT (High Pressure, High Temperature), when the initial product temperature is 70–90 °C, the process temperature is 110–120 °C and the holding time is 1 to 10 min. This method can also inactivates bacterial spores [16,17].

3. Fruit Preparations

Different types of fruit preparations are regulated by EU Council Directive 2001/112 [18] (for fruit juices and certain similar products) and Directive 2001/113 [19] (fruit jams, jellies, marmalades and sweetened chestnut purée) intended for human consumption. Following Directive 2001/112, juice is a drink that naturally contains fruit and vegetables. Juice types appearing in EU legislation are defined below: Fruit juice is juice obtained directly from fruit. The juice is not concentrated or reconstituted from concentrated juice. Fruit juice from concentrate: is juice which has been concentrated (by evaporation under reduced pressure to reduce its volume) and returned to its original state by the addition of water. Concentrated fruit juice: is juice obtained from one or more kinds of fruit juice by the physical removal of a specific proportion of the water content of the juice. Fruit nectar: is a product made by combining fruit juice, fruit juice from concentrate, concentrated fruit juice, dehydrated/powdered fruit juice, fruit puree or a mixture of these products with water and adding sugar and/or honey and/or sweeteners.

On this sector of juices and beverages, the pressure ranges between 400 and 600 MPa, and is typically applied from a few seconds to 5 min, at refrigerated or at room temperature. Specifically, the most frequently used treatment conditions for juice preservation involves 500–600 MPa and 2–3 min holding time [20]. According to the council EU Directive 2001/113 [19]: Jam is a mixture, brought to a suitable gelled consistency, of sugars, the pulp and/or purée of one or more kinds of fruit and water. However, citrus jam may be obtained from the whole fruit, cut into strips and/or sliced. Marmalade is a mixture, brought to a suitable gelled consistency, of water, sugars and one or more of the following products obtained from citrus fruit: pulp, purée, juice, aqueous extracts and peel. Compote is a recipe consisting of some sort of fruit, fresh or dried, that has been stewed in a syrup of sugar and other flavorings. The fruit in compote can be whole or puréed. When compote is made with dried fruit, the fruit is typically first soaked in water.

Some other fruit preparations as smoothies or other commercial denominations as premium juices or bar juices do not have a legal definition in the EU and there is no standard method of manufacturing. However, fruit smoothies usually contain crushed fruit, purees, and fruit juice [21]. Other derivatives such as soups, sauces, slices, and prepared dishes can be also treated by HPP technologies, although

studies found from literature review are scarce. Specifically, some authors have recently reviewed aspects related to edible flowers as broccoli and cauliflower [22].

Over the last years, several companies producing and distributing fruit and vegetable based beverages have been appearing in the EU. Table 1 and on Figure 2 are summarizing data related to these firms.

Table 1. Brands, webpages and fruit/vegetable products treated by HPP in the European Union (EU) market.

Brand	Product	Web Pages
Coldpress	Juices	http://www.cold-press.com/
Créaline	Purees, Soups	http://www.crealine.fr/
Evolution	Juices, Smoothies	www.evolutionfresh.com/
Fresh nutribits	Juices	http://www.nutribits.com
Fruity line	Juices, Smoothies	http://fruity-line.nl/en/
Hoogsteger	Juices	http://www.hoogsteger.nl/
In Fruit	Juices, Smoothies	http://www.infruit.fr/
Invo	Coconut water	https://www.invoconutwater.com/
La fruitière du Val Evel	Purees	http://www.lafruitiere.com/red-fruits.html
Presha fruits	Juices	http://www.preshafruit.com.au/cold-pressed
Press & Reset	Juices	http://www.pressandreset.com/
Romantics	Juices, Smoothies	https://www.shopromantics.es/
Teresa’s juicery	Juices, Purees	https://teresajuicery.com/
The juicy group	Juices, Smoothies	http://www.juicygroup.be/
Ulti daregal	Juices	http://www.daregal.fr/en/home/

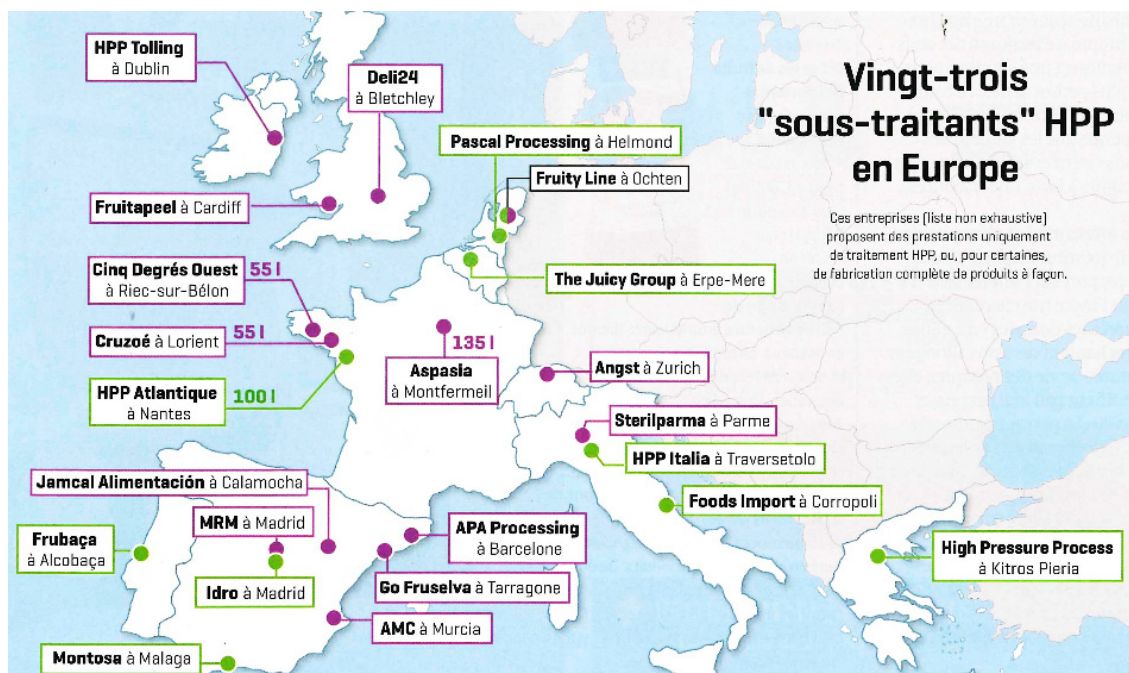


Figure 2. Placement of factories related to fruit/vegetable based beverages and other preparations in the EU [23].

Some firms choose installations with HPP units to treat their products. These machines are provided by businesses with pressurization equipment able to work at an industrial scale, usually with vessels bigger than 50 L. Therefore, some firms can process high amounts of product/hour in these units. Some examples of these firms are Hyperbaric and C-Tec Innovation.

Food factories making fruit products in the EU market are subjected to: Commission Regulation (EC) n° 852/2004 [24] on the hygiene of foodstuff and Commission Regulation (EC) n° 2073/2005 [25] concerning the applicable microbiological criteria in foodstuffs. Regarding to Regulation 852/2004, foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. This regulation was modified by Commission Regulation 1441/2007 [26]. All of them are summarized in Table 2.

Table 2. Food safety criteria in the EU legislation for fruit/vegetable preparations.

Category of Foodstuff	Microorganisms/Toxins, Metabolites	Sampling Plan ¹		Limits ^{2,3}		Reference Analysis Method	Application of Criterion
		<i>n</i>	<i>c</i>	<i>m</i>	<i>M</i>		
1.19 Fruits and vegetable precut (ready to be consumed)	<i>Salmonella</i>	5	0	Absence in 25 g		EN/ISO 6579	Product placed on the market during their shelf life.
1.20 Fruits juice and vegetable unpasteurized	<i>Salmonella</i>	5	0	Absence in 25 g		EN/ISO 6579	Product placed on the market during their shelf life.
2.5.1 Fruits and vegetable precut (ready to be consumed)	<i>E.coli</i>	5	2	100 cfu/g	1000 cfu/g	ISO 16649-1 or 2	Improvement of production hygiene and selection of raw materials.
2.5.2 Fruits juice and vegetable unpasteurized (ready to be consumed)	<i>E.coli</i>	5	2	100 cfu/g	1000 cfu/g	ISO 16649-1 or 2	Improvement of production hygiene and selection of raw materials.

¹ *n*: Number of units making up the sample; *c*: Number of sampling units giving values between *m* and *M*; ² Point 1.1 to 1.25: *m* = *M*; ³ cfu = colony-forming unit.

The safety of food products is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles. Microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. It is therefore appropriate to set microbiological criteria defining the acceptability of the processes, and also food safety microbiological criteria, setting a limit above which a food product should be considered unacceptably contaminated with the micro-organisms for which the criteria are set.

One criterion is related to *Listeria monocytogenes*. The opinion recommended by law is to keep the concentration of *Listeria monocytogenes* in food below 100 cfu/g.

The BIOHAZ (Scientific Panel on Biological Hazards) Panel of EFSA (European Food Safety Authority) issued an opinion on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs on 26 and 27 January 2005. It concluded that one of the major control measures is to control temperature and to establish a system based on hazard analysis and critical control point principles.

Nevertheless, there is no regulation in the EU about pasteurization schedules exerted by pressurization processes. The factories choose the schedule according to bibliographic data and following Research and Development tests. The factories then have to validate their schedule.

Thus, food business operators should decide themselves the necessary sampling and testing frequencies as part of their procedures, based on HACCP principles and other hygiene control procedures.

Regarding USA requirements, the Food and Drug Administration (FDA) recommends a 5-log reduction of the “pertinent micro-organism”, which is the most resistant micro-organism, in orange juice *Escherichia coli* O157:H7 (FDA, 2004) [27]. As a guideline, the FDA recommends a minimum

temperature-time equivalent for juice of 71.1 °C for 3 s for products with a pH in the range of 3.6–4.0. However, this specific temperature-time is insufficient to inactivate spoilage organisms [28].

Some reviews have been reported to expose and summarize virus inactivation processes and mechanisms in foods. However, studies reviewing effects in virus inactivation in foods treated by HPP are scarce [29]; more rare are studies dedicated specifically to vegetable and/or fruit preparations [30–32]. In some viruses, the conventional fecal indicators are unreliable for demonstrating their presence or absence. Therefore, the reliance on fecal bacterial indicator removal for determining virus inactivation is an unsafe practice.

Fruit preparations are susceptible to microbial spoilage and thus have a limited shelf life. Fruit- and/or fruit juice-borne disease outbreaks or spoilage problems have been reported primarily in the last years. However, HPP inactivation of yeast and vegetative bacteria in fruit is very effective because of low pH in fruit [33]. Indeed, microbial spoilage of fruit products such as juices may lead to off-flavors, odors, turbidity and gas production. Pathogens do not grow in fruit juices due to their low pH, but can survive and become adapted to the acidic environment.

To gain good results and assure microbial spoilage criterion, it is important to know about the HPP inactivation of pathogens with an adequate margin of safety, and how pressure-time-temperature combinations affect the inactivation.

The type of fruit/vegetable preparations more commonly processed by HPP as reported in the reviewed literature was juices. As far as we know, no studies were found to be related to Marmalade and Compote preparations treated by HPP. This fact could be attributed to the low water activity of these last derivatives that make these products inappropriate for this preservation treatment.

4. Effects on Microorganisms and Viruses

Microbial inactivation is one of the main goals for the application of high pressure technology [9]. So, some processes, factors and mechanisms are exposed below in order to describe how HPP prevents microbial growth in foods.

Microbial inactivation in foods is affected by many characteristics, such as microorganism taxon (gender, species, strain), food treatment, food group (animal, vegetal), processing type (fresh, processed) and food structure. For instance, food structure is a main factor affecting microorganism proliferation and shelf life extension. The properties that contribute to food structure are: mechanical distribution of water, chemical distribution of food preservatives, and physical constraints on mobility of microorganisms [34].

Regarding to pressurization effects in food matrices, HPP leads to modifications of cellular membranes and interrupts cellular functions which are responsible for reproduction [35]. Those are one of the main causes of bacterial death. The pressure also plays a role on the availability of the energy within the cells, because it affects some biochemical reactions which produce energy. It can also affect certain molecular reactions, such as genetic expression and protein synthesis, between 30 and 50 MPa.

In general, HPP above 200 MPa inactivates vegetative bacteria, yeast, and molds. In practice, pressures up to 700 MPa and treatment times from a few seconds to several minutes are used to inactivate microbial cells. Bacterial spores on the other hand, are highly resistant to pressure, showing a remarkable tolerance to pressures above 1000 MPa near room temperature. Nevertheless, sterilization of low-acid foods, as some fruit derivatives, is possible through combined high pressure (500–900 MPa) and relatively mild temperature (90–120 °C) processing for about five minutes [36].

With respect to microorganisms and virus types, many studies showed that the HPP treatment does not work the same way for all taxa [37,38]. Indeed, different species can have varying pressure resistance and the stage of growth of bacteria is also important in determining pressure resistance: for example, cells in stationary or dormant phase are more pressure resistant than those in the exponential phase. Gram-positive bacteria are more resistant than Gram-negative bacteria [39], cocci

are more resistant than rods. Many studies related to specific microorganisms can be found in the literature [40,41].

Bacterial spores are very resistant to pressure [42]. Butz et al. (1990) [43] investigated the effects of pressures between 150 and 400 MPa at temperatures of 25 to 40 °C on bacterial spores, and showed that pretreatment at relatively low pressures (60–100 MPa) led to accelerated inactivation of spores at high pressure. Several papers on the use of HPP to inactivate spores have made similar suggestions for a two-exposure treatment with HPP to enhance the inactivation of spores. The first exposure germinates or activates the spores, and the second exposure at a higher pressure inactivates the germinated spores and vegetative cells [44]. Some reviews have analyzed the inactivation in vegetative cells, virus and spores [45].

Concerning yeast and mold, these are less resistant than bacteria [37]. Indeed, they are inactivated by pressure between 200 and 400 MPa. Most yeast and mold spores are destroyed by a pressure of 400 MPa, as shown in Table 3. However, *Saccharomyces cerevisiae* seem to be more resistant than Gram-negative bacteria [42]. Basak et al. (2002) [46] demonstrated that *S. cerevisiae* was not inactivated under a pressure equal to 400 MPa in orange juice. It was shown that the resistance of these microorganisms increased when the concentration of sugar in the environment also increased [47]. This can cause a problem during the treatment of the fruit-based preparations containing a strong concentration in sugars.

Table 3. Microbial safety (expressed in log reductions) of vegetative forms (bacteria, yeast and molds) and shelf life, in fruit/vegetable preparations processed by HPP.

Fruit/ Vegetable	Product Type	Reference		HPP Conditions	Microbial Inactivation Criterion	Log Reductions *	Shelf Life (Storage T°)
		Author, Year	No.				
Apple	Puree	Landl, 2010	[48]	400–600 MPa 15 min 20 °C	Aerobic mesophilic bacteria	3.3	14–21 days 4 °C
					Yeasts/Molds	3.2	
Apple	Juice	Shahbaz, 2016	[40]	500 MPa 1 min 25 °C	<i>L. monocytogenes</i>	4.8	NP
					<i>S. aureus</i>	2.4	
					<i>E. coli</i>	5.0	
					<i>S. tiphimurium</i>	7.0	
Apple Orange	Juices	Hartyáni, 2013	[49]	200–600 MPa 10 min 20–60 °C	<i>Alicyclobacillus</i> <i>acidoterrestris</i>	2.2	14–28 days 4 °C
						2.0	
Apple	Juice concentrate	Lee, 2006	[50]	207–621 MPa 5–10 min 22–90 °C	Spores of <i>Alicyclobacillus</i> <i>acidoterrestris</i>	2–5	NS
Apple/ Plum	Jams	Prestamo, 1999	[51]	100–400 MPa 5–15 min 5–20 °C	<i>L. monocytogenes</i>	1–9	NS
Apple/ Apricot	Juices	Bayindirli, 2006	[41]	350 MPa 5 min 30 °C	<i>S. aureus</i>	6.8/6.9	NP
					<i>E. coli</i>	7.1/7.3	
Sour Cherry/ Orange					<i>S. enteritidis</i>	7.8/8.0	
					<i>S. aureus</i>	7.4/7.3	
					<i>E. coli</i>	7.7/7.4	
Banana	Smoothie N ₂ -degassed	Li, 2015	[52]	550 MPa 2–10 min 20 °C	Total aerobic bacteria	2	>15 days 4 °C
					Yeasts/Molds	2.5	

Table 3. Cont.

Fruit/ Vegetable	Product Type	Reference		HPP Conditions	Microbial Inactivation Criterion	Log Reductions *	Shelf Life (Storage T ^a)
		Author, Year	No.				
Mung Bean Sprout	Juices	Muñoz, 2008	[53]	150–400 MPa 2 min 20–40 °C	Aerobic mesophilic bacteria Fecal coliforms	1.7–5.7 up to 7.8	>13 days 4 °C
Sour Chinese Cabbage	Fresh	Li, 2010	[54]	400–600 MPa 10–30 min rt	Total aerobic bacteria Lactic acid bacteria Yeasts	2.7–4.5 2.4–7 1.5–4.2	60 days 4 °C
Cactus	Juice	Moussa, 2017	[55]	600 MPa 10 min 15 °C	Viable microbial cells Yeast/Molds Acid tolerant microorganisms	3	NP
Cantaloupe	Puree	Mukhopadhyay, 2017	[56]	300–500 MPa 5 min 8 °C, 15 °C	Total aerobic bacteria	1–3.3	10 days 4 °C
Carrot, Melon or Papaya	Soy Smoothie	Andrés, 2016	[57]	550–650 MPa 3 min 20 °C	NP	NP	45 days 4 °C
Cashew apple	Juice	Lavinas, 2008	[58]	250–400 MPa 3–7 min 25 °C	Aerobic mesophilic bacteria Yeasts/ Filamentous fungi <i>E. coli</i>	1.8–5.9 NP 6.4–6.5	56 days 4 °C
Cherimoya	Pulp	Perez, 2015	[59]	600 MPa 8 min 20 °C	Viable microbial cells	5	7–15 days
Cucumber	Juice	Zhao, 2013	[4]	500 Mpa 2 min	Viable microbial cells Yeasts/Molds	2 3–4	50 days 4 °C
Feijoa	Puree	Duong, 2015	[60]	200–400 MPa 6 min 25 °C	<i>E. coli</i> <i>B. subtilis</i> <i>S. cerevisiae</i>	2.5 2.5 6.5	NS
Red Grape White Grape	Juices	Mert, 2013	[61]	150–250 MPa 5–15 min, 20–40 °C	Viable microbial cells	5 7	90 days 20 °C (dark storage)
Keiskei	Juice	Chai, 2014	[62]	550 MPa 1.5 min rt	Coliform bacteria Yeasts/Molds <i>Pseudomonas</i> <i>B. cereus</i>	6.0 4.8 5.3 2.3	6 days 4 °C
Kiwi	Puree	Fernández-Sestelo, 2013	[63]	500 MPa 3 min rt (20–25 °C)	Viable microbial cells	3.4	21 days 4 °C
Kiwi/ Pineapple	Juices	Buzrul, 2008	[64]	350 Mpa 5 min 10–20 °C	<i>E. coli</i> <i>L. innocua</i>	~5.5/~2.5 ~5.5/~3.5	21 days 4 °C–37 °C
Mandarin	Juice	Carreño, 2011	[65]	150–450 MPa 0,1–1 min 15–45 °C	<i>L. plantarum</i>	0–1	NS

Table 3. Cont.

Fruit/ Vegetable	Product Type	Reference		HPP Conditions	Microbial Inactivation Criterion	Log Reductions *	Shelf Life (Storage T ^a)
		Author, Year	No.				
Mango	Nectar	Liu, 2014	[66]	600 MPa 1 min 20 °C	Total aerobic bacteria Yeasts/Molds	5.2 3.1	NS
Mango	Pulp	Liu, 2013	[67]	300–600 MPa 1–20 min °C NP	Total aerobic bacteria Yeasts/Molds	3.8 1.5–2.0	NS
Olive	Jam	Delgado-Adamez, 2013	[68]	450/600 MPa 5 min 10 °C	Aerobic mesophilic bacteria Yeasts/Molds <i>E. coli</i>	ND	>6 months
Orange	Juice	Bull, 2004	[69]	600 MPa 1 min	Total aerobic bacteria Yeasts/Molds	4.5 3.5	56 days 10 °C
Orange	Juice	Katsaros, 2010	[70]	100–500 MPa 1–30 min 20–40 °C	<i>L. plantarum</i> <i>L. brevis</i>	0.2–4.5 0.1–4.0	NS
Orange	Juice	Linton, 2015	[71]	400–550 MPa 5 min 20–30 °C	<i>E. coli</i>	5–6	NS
Orange	Juice	Polydera, 2004	[72]	600 Mpa 4 min 40 °C	NS	NS	187–147 days 0 °C
Orange	Juice	Syed, 2014	[73]	700 Mpa 5 min 4 °C	<i>S. aureus</i>	6.2–6.6	15 days 4 °C
Orange	Juice	Timmermans, 2011	[28]	600 Mpa 1 min 17 °C	Viable microbial cells Yeasts/Molds	3–8 3–5	58 days 4 °C
Orange	Juice	Yoo, 2015	[74]	400 Mpa 1 min	<i>E. coli</i>	2.4	NP
Orange, Apple or Tomato	Juices	Jordan, 2001	[75]	500 MPa 5 min rt (20 °C)	<i>E. coli</i>	~5	NP
Orange/Peach	Juices	Erkmen, 2004	[76]	200–700 MPa 1–90 min 25 °C	Total aerobic bacteria <i>L. monocytogenes</i>	7.8/7.5 7.8/7.5	NS
Pear	Nectar	Guerrero, 2011	[77]	103–241 MPa 2 s–15 min 25 °C	<i>E. coli</i> <i>L. innocua</i> <i>S. cerevisiae</i>	0.1–1.5 0.1–0.2 0.1–0.4	NS
Sauerkraut	Fermented cabbage	Peñas, 2010	[78]	300 MPa 10 min 40 °C	Total coliforms Aerobic mesophilic bacteria Lactic acid bacteria Faecal coliforms Yeasts/Molds	<1 4.2 4.2 <1 <1	60 days 4 °C

Table 3. Cont.

Fruit/ Vegetable	Product Type	Reference		HPP Conditions	Microbial Inactivation Criterion	Log Reductions *	Shelf Life (Storage T ^a)
		Author, Year	No.				
Soy	Smoothies	Andrés, 2016	[57]	450–650 MPa 3 min 20 °C	Aerobic mesophilic bacteria	3	30 days 4 °C
Strawberry	Puree	Marszalek, 2015	[79]	300–500 MPa 1–15 min 50 °C	Viable microbial cells Yeasts Molds	1.5–2.4 2.6–3.6 0.5–3.8	NS
Tomato	Juice	Hsu, 2008	[80]	300–500 MPa 10 min 25 °C	Viable microbial cells Yeasts/Molds Enterobacteria Lactic acid bacteria	0.9–4.1 3.7/3.6 2.1 4.2	28 days 4 °C
Tomato	Puree	Krebbbers, 2003	[81]	700 MPa, 0.5–2 min 20–90 °C	<i>B.</i> <i>stearothermophilus</i>	2.7–6.1	56 days 4 °C
Tomato/Water melon	Juice	Aganovic, 2017	[82]	600 Mpa 5 min	Collected from literature	5	NP
Red fruits with orange, banana + lime	Smoothies	Hurtado, 2017	[83]	350 MPa 7 min <25 °C	Aerobic mesophilic bacteria Psychrotrophic bacteria Yeasts/Molds Enterobacteria	1.8 2.5 1.8 2.4	28 days 4 °C
Wild berries with grapes, apples + orange	Smoothies	Scolari, 2015	[84]	100–300 MPa 5 min 5–45 °C	<i>L. monocytogenes</i>	2–6.3	NS

*: log Reductions are globally taken from the most lethal conditions. NP: not provided; NS: not studied, rt: room temperature.

Table 3 summarizes inactivation data (expressed in microbial log reductions) and shelf life related to fruit and vegetable based products treated by HPP on vegetative microorganism, yeast and molds.

Table 3 is a review of the contents of 42 works, from 1999 up to May 2017. The fruit juices are the preparations most studied (57%), followed by purees/pulp (15%), and smoothies (12%). Despite jams having been the first pressurized fruit preparations, only two studies related to microbial inactivation in these types of products were found in the revised literature, and none considered compote or marmalade. This was probably conditioned by their low water content and the preservation induced by their high sugar content.

Regarding the type of fruit, oranges and apples were the most studied. With regard to inactivation effectiveness, many factors can influence the spoilage reduction of various microorganisms, as commented before. Mild conditions (i.e., 100 MPa, <5 min at room temperature can reach only one log reduction or less in *L. monocytogenes*, total aerobic bacteria, *L. plantarum*, *L. brevis*, *E. coli*, *L. innocua*, *S. cerevisiae*, faecal coliforms, and yeast and molds [51,56,65,70,77–79]. Increasing the pressurization conditions (>350 MPa, 5 min and >20 °C), inactivation > 7 log reduction can be achieved for certain microorganisms [40,41,53,54,61,76]. Nevertheless, for certain apple juices, more lethal conditions only achieved up to 3, 3 log reductions in aerobic mesophilic bacteria [48], and 1 unit lower approx. for *Alicyclobacillus acidoterrestris*, [49] being necessary high temperatures to inactivate its spore [50].

With regards to estimation of shelf life, almost half of the reviewed works did not consider this parameter in their experimental plans. Therefore, more research aspects including sensory and nutritional properties need to be considered in future articles.

Currently, the literature contains few works related to the effect of the pressure on viruses. Viruses do not reproduce in food. The spread of viruses via food is mostly due to contact of food with animal excrement, human effluents, or sewage. The viruses most commonly present in food are rotavirus, norovirus, and hepatitis A-causing viruses.

It has been shown that the resistance of viruses to pressure changes greatly, is largely determined by their structure [29], and is related to the taxonomic groups or even strains, and the temperature applied during pressurization [85]. Enveloped viruses are usually more sensitive to pressure treatments than naked viruses. HPP can cause damage to the virus envelope, preventing the virus particles binding to cells or even complete dissociation of virus particles, which may be either fully reversible or irreversible, depending on the pressure. Prions, associated with neurological disorders in animals and humans, are generally even more difficult to destroy than bacterial spores [38].

A study on the hepatitis A virus showed a 7-log reduction of the infection power of this virus with a 450 MPa treatment during 5 min at 21 °C [39]. These same authors also demonstrated that the felincalicyvirus (a norovirus) was inactivated at 275 MPa for 5 min. Other studies showed that treatment requires very drastic conditions, with the application of pressure up to 1200 MPa for 10 min at a temperature up to 135 °C, to inactivate some viruses [86].

To explain and predict the behavior and inactivation of microorganisms, some mathematical models including different equations are found in the literature.

Basically, there are mainly four types of survival curves that are found for the inactivation of microorganisms: linear curves, curves with shoulder, curves with tailing, and sigmoid curves [87].

In the 1920s, the principles of thermobacteriology were established assuming that microbial inactivation has been assumed to follow a first-order reaction kinetics. This means that a logarithmic-linear behaviour is observed when survivors are plotted as a function of time at a constant lethal temperature T . This model developed in the early 1900s assumes that all microorganisms of the same strain have the same sensitivity to heat, and thus the same probability of inactivation [88].

The mathematical expressions are:

$$\log(N_t) = \log(N_0) - (t/D_T) \quad (1)$$

$$\ln(N_t) = \ln(N_0) - (t/D_T) \quad (2)$$

1. N_0 : Initial population of microorganisms.
2. N_t : Number of the survivors after the treatment.
3. t : Treatment time.

4. D_T : Decimal reduction time is defined as the time at a constant lethal temperature T , and a constant pressure to achieve one logarithmic reduction: it represents the inactivation of 90% of the microbial population of interest (Figure 3a).

5. z : Thermal resistance constant is defined as the temperature increase needed to achieve one logarithmic reduction in D_T (90% reduction), see Figure 3b, i.e.,

$$D_T + z = 0.1 D_T \quad (3)$$

6. Number of decimal reductions (S) in the microbial population of interest defined as:

$$S = \log(N_0/N_t) \quad (4)$$

An example is shown in Figure 3c.

Those parameters (mainly D_T , S and z) are well established for thermal processes but are very scarce the ones limited for the pressurization treatments. Some examples are cited by Mujica et al. (2011) [89]. Mathematical models used to analyze inactivation kinetics have already been exposed by some researchers [90].

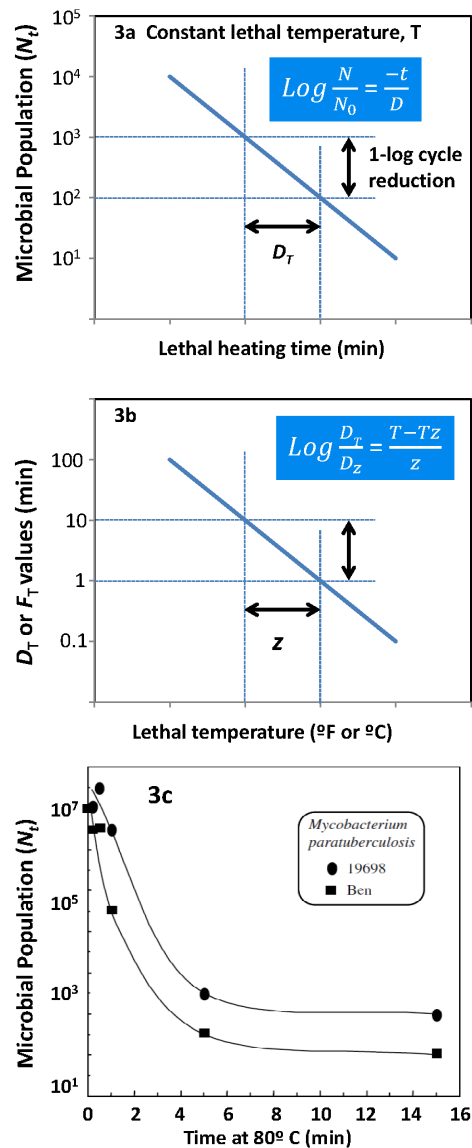


Figure 3. Microbial inactivation D-z model based on first order kinetics. (a) Decimal reduction time; (b) Thermal resistance; (c) Model deviation example. From reference [88].

The mathematical model used to describe inactivation of microorganisms was based on modifications of the Gompertz equation and described by Zwietering et al. [91]. Badhuri et al. (1991) [92] were the first to demonstrate that the modified equation of the Gompertz model describes the nonlinear survival curves of *Listeria monocytogenes* heated in sausage slurry. Later, it was successfully tested to describe bacterial heat inactivation [93].

The Weibull model considers that the microbial death probability depends on the biological variation or heterogeneity within the population of microorganisms. The Weibull type distribution of resistance model was successfully fitted to survival curves. This model is a useful tool to select the best combination necessary to achieve the inactivation of the most parts of bacterial population (pathogens). The mathematical expression is taken from Buzrul and Alpas (2004) [94]:

$$\log (Nt) / \log (N_0) = \log S = -bt^n \tag{5}$$

8. b : a rate parameter

9. n : a measure of the shape of the isothermal and of the isobaric semilogarithmic survival curve

The parameters b and n are temperature and pressure dependent.

This model was effectively applied to adjust microbial inactivation in orange juice [74] and in aguamiel from Agave Mapiaga [95]. The research group of this last reference has just reviewed this model in different food products, including mango, apple, orange and pear juices [96]. Other models such as Log-Logistic are also described by these authors [97]. This model is based on the reported data by Cole et al. 1993 [98].

5. Conclusions

The high hydrostatic pressure actually is known to have a strong development, with more than 160 industrial installations and an increase in the number of treated and marketed products, being the main sector the fruit/vegetable preparations.

This treatment is considered as an alternative way of conservation in heat treatment. Indeed, they allow to inactivate the microorganisms and to extend the shelf life of different fruit/vegetable based foods, complying, in most of them, the legal microbial requirements. It is extremely important to optimize the parameters (temperature, pressure, time) in order to assure the minimum spoilage in a fruit/vegetable product.

Some mathematical models could help in explaining and predicting the inactivation processes to make the equivalence with other preservation treatments as heat or pulsed electric fields.

There is still a lack of knowledge regarding virus inactivation in order to adapt to mathematical models and to calculate inactivation parameters as decimal reduction (D_z) and compare z values obtained in different preservation processes.

In this review, only the safety parameters could be considered in order to give an estimation of the preservation food period. Shelf life should be estimated, considering all the quality parameters affected by HPP, and the loss in nutritive value and sensory properties. This will assure the safety of the product and influence purchase decisions.

Pressurization is an emerging technology with great potential for food-processing industries. There are nevertheless certain inconveniences which are, for the moment, the cost of the investments and the resistance of the bacterial spores at the pressures applied in the food industry.

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