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Review Article

Inactivating Food Microbes by High-Pressure Processing and Combined Nonthermal and Thermal Treatment: A Review

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High-pressure processing (HPP) is a mild technology alternative to thermal pasteurization and sterilization of different food products. HPP has emerged to provide enormous benefits to consumers, i.e., mildly processed food and additive-free food. It effectively retains bioactive compounds and extends the shelf life of food commodities by inactivating bacteria, yeast, mold, and virus. The limitation of HPP in inactivating spores can be overcome by using other thermal and nonthermal processing sequentially or simultaneously with HPP. This review summarizes the applications of HPP in the fruits and vegetables, dairy, meat, fish, and poultry sector. It also emphasizes microbial food safety and the effectiveness of HPP in the load reduction of microorganisms. Comprehensive information about the synergistic effect of HPP with different techniques and their effectiveness in ensuring food safety is reported. The summarized data would be handy to interested researchers and industry personnel.

1. Introduction

Most food commodities, i.e., fruits, vegetables, meat, poultry, seafood, milk, and their products, are perishable due to limited shelf life. The presence of moisture and environmental conditions (temperature and relative humidity) around food products during storage triggers physical, chemical changes, and microbiological growths leads to food deterioration or spoilage [1]. Spoilage can be defined as undesirable changes that render a product unsuitable for consumption due to physical, chemical, or microbiological changes. Physical changes include loss of moisture from dried foods, gain of moisture, and freeze burn. In many cases, physical changes in food products during storage also lead to chemical reactions and microorganism growth. Some undesirable chemical changes are staling, discolorations (by enzymatic browning and nonenzymatic browning), off-flavor development (due to oxidation of food leading to rancidity), etc. in the food products [2]. These chemical changes or reactions can be triggered by specific enzymes, i.e., lipases, peroxidases, polyphenol oxidases, and catalases. These changes reduce food quality and acceptability by consumers and are not recommended for consumption. Microbial spoilage can occur when bacteria, mold, and yeast grow in food or produce toxins harmful to humans. Apart from storage temperature and relative humidity, the growth of microorganisms in the food also depends on food composition. Since food is a rich source of nutrients and

water activity of most of the fresh commodities is high. So, it provides a very suitable environment for the microorganism to thrive on food. Food deterioration by the growth of pathogenic microorganisms is a significant concern [2]. As pathogenic bacteria are linked with outbreaks of fruits, vegetables, dairy, meat, and poultry-based products. Taiwan Food Drug Administration reported more than 2000 cases of food poisoning due to accidental consumption of fresh fruits, vegetables, and seafood products contaminated with pathogenic microbes [3]. So, it is important to process food commodities to keep them safe and as well as to extend their shelf life.

Different processing techniques (i.e., thermal and nonthermal technologies) are used to prevent physical contaminations, slow down the chemical, enzymatic reactions, and eliminate/reduce microbial spoilage. Although thermal processing (which involves applying heat) is the most used and is a well-established treatment in terms of its historical use, predictability, and cost, it impacts the nutritional quality due to the high temperature involved while processing. Nowadays, nonthermal techniques (which involve applications of pressure, short pulse electric field, light, and sound waves) are preferred over thermal techniques. As the temperature attained by the product during nonthermal processing is low, lower nutritional losses occur during food processing [4]. Moreover, consumers prefer minimally processed food with a clean label and products processed with nonthermal techniques. High-pressure processing (HPP), pulse electric field, irradiation, cold plasma, and ultrasound are some of the nonthermal processing techniques. Among these nonthermal techniques, HPP in compliance with consumer requirements, provide products similar to fresh, clean label, additives-free, and convenient with extended shelf life. It is a promising cold pasteurization technology and gaining importance worldwide [5–7].

For commercial processing, HPP utilizes the application of high pressure (100–600 MPa) for a particular time at room temperature to packaged food kept inside a vessel to inactivate microorganisms. The vessel contains solvent (i.e., water, propanol ethanol, etc.), which transmits the pressure equally and uniformly throughout the vessel [8]. HPP of food commodities leads to changes in the cell membrane, cell morphology, biochemical reactions, and alteration in the genetic mechanism responsible for microorganism inactivation. These effects vary with the type of microorganism and food composition. Hence, it is of utmost importance to optimize the process parameters (pressure/holding time/temperature) to ensure food safety with adequate margins [9].

Food with low pH values (pH values < 4.6) indicating high acid intensity is less likely to be spoiled by bacteria. But, low acid foods (pH values > 4.6) are less microbiologically stable, and bacteria tend to produce a dormant form known as spores in fresh food [10]. Spores are tough to kill/inactivate, and if they are not appropriately inactivated, they wait for favorable conditions to grow [11]. So, it is of utmost importance to kill spores. HPP at room temperature is not adequate to inactivate all the spores, especially in the case of low-acid foods. To achieve a higher efficacy in inactivating pressure-resistant pathogens and spores, temperature (90–120°C) can simultaneously be increased during HPP. The combination of heat (90–120°C) with pressure sequentially or simultaneously has been reported to provide synergistic effects against spores on different food commodities [12]. Similarly, sequential use of other techniques like irradiation, preservatives along with HPP has shown synergistic and additive effects against spores/pathogens to achieve food commodities safety [13–15].

Keeping food safety in view, the main objective of this review is to summarize the existing data from the published research concerning the effects of high-pressure treatment to inactivate microorganisms in fruits and vegetables; milk and milk products; meat, poultry, and seafood, and their products. Sometimes, high pressure alone is ineffective for the complete inactivation of pathogens and spores; therefore, a different strategic combination of thermal and nonthermal techniques assisting high pressure is also assayed. The provided information would be beneficial to interested researchers and industry personnel.

2. High-Pressure Inactivation in Specific Food Sector

The effectiveness of treatment to inactivate microbial populations depends on the type of microorganism, species, types of food (plant or animal origin), and matrix of food. The lethal effect of HPP on microbial population is assumed to be due to simultaneous effects on cell membrane permeability, changes in cell morphology, altered biochemical reactions, interference in the genetic mechanism, which occurs in the cell of microorganism, and detailed mechanism has been reported by Sehrawat et al. [16].

2.1. Fruits, Vegetables, and Their Products. Fresh agricultural products are healthy and nutritious, but contamination by microorganisms has been reported during storage. Second, there is a huge demand for refrigerated stored salad, fresh-cut fruits, and vegetables available in the market. Apart from being healthy, the availability of these products from the market saves time and provides convenience to the customers. But during cutting and packaging, chances of growth of *E. coli* O157: H7 and *Salmonella* might occur. Recently, frequent food-borne pathogens outbreaks are linked with these products and are of major public health concern. Contamination of raw products with pathogenic microorganisms can be due to their direct growth or indirect sources such as insects, water, and soil. HPP has been proven to be an effective technology for eliminating these pathogens of concern [17]. The effectiveness of HPP in reducing the microbial population is given in Table 1.

Apart from providing microbial safety, another reason for considering HPP as an alternative to convention preservation techniques is its limited effects on covalent bonds resulting in an only minor modifications in nutritional and sensory aspects. Pressure-treated juices are now available on a commercial scale in many countries viz. France

Table 1: Effect of HPP to inactivate microorganisms in fruits, vegetables, and their products.

Product	Microflora	Treatment (MPa/min/°C)	Log reduction*	Shelf-life (days/°C)	Optimum conditions (MPa/min/°C)	Reference
Apple cubes	*Candida lipolytica*, *E. coli*	200–650/10/25, 40	6	90/5	600/10/25	Vercammen et al. [18]
Litchi fruits	Total aerobic mesophilesY&MPsychrotrophs	100–300/5–15/27	3.293.243.77	32/5	300/10–15/27	Kaushik et al. [19]
Orange comminuted	Aerobic bacteria	100–400/1–4/1–9	3–5	—	414/2/—	Serment-Moreno et al. [20]
Green beans	Total plate count	500/1/20	4	30/6	500/1/20	Krebbers et al. [21]
Sour Chinese cabbbage	Lactic acid bacteria (LAB)	200–600/10–30/25	6-7	60/4	600/10/25	Li et al. [22]
Sauerkraut	Aerobic mesophilic bacteria, LAB, coliforms	300/10/40	4-5	90/4	300/10/40	Penas et al. [23]
Green onion (soaked)	*Salmonella*; *E. coli* O157: H7	250–500/2/20–40	>5	15/4	400–450/2/20–40	Neetoo et al. [24]
	Salmonella enterica; *E. coli* O157: H7	300–500/2/20–40	>5	—	400/2/40	Neetoo et al. [25]
Carrot, spinach	*Salmonella typhimurium*	100–500/0–20/30	>5	—	500/5/30	Jung et al. [26]
Radish	Total plate countY&M	300–550/5/	5.57	90/4	550/5/-	Bao et al. [27]
Tomato puree	Total plate count	50–400/15/25	4	—	400/15/25	Plaza et al. [28]
Mango puree	*Saccharomyces cerevisiae*	207–552/5–15/25	5	27/3	552/5/25	Guerrero-Beltran et al. [29]
Granny smith apple puree	Total aerobic mesophiles; Y&M	400–600/5/20	3	21/5	400/5/20	Landl et al. [30]
Cantaloupe puree	Aerobic plate count, Y&M	300–500/5/8–15	3	10/4	400–500/5/15	Mukhopadhyay et al. [31]
Plum puree	Total aerobic mesophilic, Y&M	400–600/7/10	1-23	20/4	600/7/10	González-Cebrino et al. [32]
Mango pulp	Y&M	100–600/0–20/30	4.6	—	600/5/30	Kaushik et al. [33]
Orange juice	*Salmonella*	600/1/20	7	—	600/1/20	Teo et al. [34]
Valencia and navel orange juice	Total aerobic bacteria, Y&M,	600/1/20	>7>4	84/4	600/1/20	Bull et al. [35]
Orange juice	Aerobic plate count, Y&M	600/1/—	5–83–5	58/4	600/1/—	Timmermans et al. [36]
Cashew apple juice	*E. coliAerobic mesophiles*Yeast and fungi	250–400/3–7/25	643	56/4	400/3/25	Lavinas et al. [37]
Kiwifruit and pineapple juice	*E. coli*; *L. innocua*	300–375/(0–5) × 2–10 pulses/−10, 0, 20	7	21/4, 7, 37	350/1 × 5 pulses/20	Buzrul et al. [38]
Pomegranate juice	Total plate count	400–600/5–10/25–50	4	—	400/5/25	Ferrari et al. [39]
Cantaloupe juice	Total plate count, *E. coli, Bacillus subtilis*	400–500/0–20/22	453	—	500/20/22	Ma et al. [40]
Mango juice	*L. mesenteroides, E. coli* O157: H7	250–550/0–60/20–23	6	28/4, 12, 20	500/1/-	Hiremath and Ramaswamy [41]
Papaya beverage	Total plate count, Y&M	350–650/5–10/-	43	40/4	350/5	Chen et al. [42]
Pomegranate juice	Aerobic mesophiles, Y&M	350–550/0.5–2.5/4	4	35/4	350/2.5/5	Varela-Santos et al. [43]
White grape juice	Aerobic plate count, coliforms, Y&M	300–600/3/20	21	20/4	600/3/20	Chang et al. [44]
Elephant apple juice	Total viable bacteria, Y&M	600/5/35	3-4	60/4	600/5/35	Nayak et al. [45]
Apple-brocooli juice	*Saccharomyces cerevisiae), Aspergillus flavus, E. coli*	250–500/5–20/15	>5	30/5	500/10/15	Houška et al. [46]

Table 1: Continued.

Product	Microflora	Treatment (MPa/min/°C)	Log reduction*	Shelf-life (days/°C)	Optimum conditions (MPa/min/°C)	Reference
Apple, orange, and tomato juices	*Alicyclobacillus acidoterrestris*	350/20/50	4	21/30	350/20/50	Alpas et al. [47]
Carrot juice	*E. coli* O157: H7*Staphylococcus aureus*	200–400/0–15/40	55	—	—	Pilavtepe-Çelik et al. [48]
Carrot juice	Total plate count*L. monocytogenes*	500–600/1/20	46	22/4	500/1/20	Patterson et al. [49]
Wheatgrass juice	*E. coli* P36, *Listeria innocua* ATCC 51742, and *S. typhimurium* WG49	400–600/1–3/11	>5	—	500–600/1/11	Ali et al. [50]
Olive jam	Coliform; *Bacillus cereus, Salmonella; L. monocytogenes*	450–600/5/10	ND	540/4	600/5/10	Delgado-Adamez et al. [51]
Purple sweet potato nectar	Total aerobic bacteria, Y&M	400–600/2.5–10	64	84/4	—	Wang et al. [52]
Mango nectar	*E. coli*Mesophiles	275–414/0–5/17	7-87	——	414/2/17 or 315/4/17414/4/17	Aguirre et al. [53]

ND = not detected; *log reductions are cited from the most lethal parameters after HPP treatment.

(Ultrifruit), Japan (Waka Food Industries), Portugal (Frabaca), the UK (Orchard House), the USA (Odwala), and Mexico (Grupo Jumex).

Different fruits and vegetables that have been processed using high-pressure are apples, litchi, orange, papaya, pomegranate, kiwi, plum, pineapple, cashew apple, green beans, cabbage, radish, carrot, spinach, wheat grass, onion, etc.

Different microflorae require different pressure treatments in order to inactivate them. Pathogenic *E. coli* was the most reported to be a more resistant bacterial strain than *Listeria monocytogenes* in mango juice to high-pressure treatment [41]. Around 6 log reduction of *E. coli* O157: H7 and 5 log reduction of *L. monocytogenes* were achieved at 400 MPa for 10 min and 500 MPa for 1 min; there were no survivors of *E. coli*. For *Z. bailii, P. membranaefaciens*, and *L. mesenteroides,* the pressure of 300 MPa was sufficient to reduce the count to less than 1 log CFU/mL [41]. So, the most resistant microorganism can be selected as the target microorganism to get the optimum conditions for the treatment. Pilavtepe-Çelik et al. [48] reported the inactivation of *E. coli* O157: H7 and *Staphylococcus aureus* by high-pressure treatment (200–400 MPa/0–40 min/40°C) in carrot juice and peptone water. The carrot juice medium showed pressure resistance to *E. coli* (add pressure treatment), whereas *S. aureus* (add pressure treatment) was more resistant to peptone water than the carrot juice medium. This specific effect on *S. aureus* is due to the release of naturally occurring constituents of phytoalexins (6-Methoxymellein from carrot root, which is an antimicrobial compound produced with the response to microbial infection) in cellular and vascular fluids, exerting a toxic effect. It was also evident from earlier literature that this 6-methoxymellein from carrot cells was more effective against Gram-positive bacteria than Gram-negative bacteria [54]. So, it is important to consider the medium in which treatment is given as it can have varied results. Another study on carrot juice pressurization at 500 and 600 MPa for 1 min at 20°C showed a significant reduction in microbial count from (4 log reduction), and a shelf life of 22 days was reported at 4°C [49]. During the storage study, at 8°C count was higher, although it took a long time to reach the maximum level, it was lower than the control samples. Pressure treatment of carrot juice at 500 MPa/1 min/20°C followed by storage at 8°C, for 22 days inactivated the competitive microflora except for spore formers and *L. lactis* (non-spore former) [49]. It can be concluded that apart from the amount of pressure, duration of treatment, and storage temperature; the type of microorganism plays an important role.

The juice of wheatgrass was given different treatments, i.e., thermal (75°C/15 s), HPP (500 MPa/60 s), and ultraviolet-C light (254 nm/69.2 mJ/cm^2) to achieve 5 log CFU reduction of microorganisms. Although all the treatment conditions mentioned above were found to be effective in the inactivation of microorganisms like *E. coli* P36, *L. innocua* ATCC 51742, and *S. typhimurium* WG49. However, thermal processing leads to a reduction in chlorophyll content, antioxidant properties, and loss of color [50]. So, HPP was reported to be the preferred method of processing as it retained maximum nutrients and gave a higher yield, and was recommended for other beverages with the same equivalent treatments. Apart from the safety of food, quality is important and is given diligent consideration by processors and consumers. Similarly, in another study by Chang et al. [44]; HPP and thermal treatment were given to white grape juice. Thermal processing (90°C/60 s) and HPP (600 MPa/3 min) were found to be effective in increasing the shelf life of white grape juice for 20 days. Differences in HPP processed juice and fresh were not significant based on sensory analysis, but thermally processed juice showed low acceptance [44]. The initial population of aerobic plate count, Y&M, and coliform count for control juice were 3.2, 2.2, and 2.1 log CFU/mL. When compared to treatment at 300 MPa/3 min, 600 MPa/3 min showed a significant

reduction in aerobic plate count by more than 2.0 log CFU/mL, yeast and mold (Y&M), and coliforms to <1 log CFU/mL, respectively. The microbial reduction (600 MPa/3 min) was similar to the effect of traditional thermal treatment. At 20 days of storage, the control sample showed aerobic plate count Y&M, and CC as 4.9, 3.3, and 2.3 log CFU/mL, whereas HPP treatment at 600 MPa/3 min showed very low aerobic plate count and minimum detection limit of Y&M and coliforms. Besides, HPP preserves the color and odor of juices by mitigating the Maillard reaction that generally occurs in traditional thermal processing [44].

HPP treatment (600 MPa/5 min/35°C) of elephant apple juice extended the shelf life of juice by 60 days at 4°C (microorganism count was <1 CFU/mL), whereas the microbial count was higher in thermally processed and control samples during the shelf-life study. The untreated samples showed a continuous increase in microorganism number during the storage study and were unacceptable by the end of 10 days as total viable bacteria; Y&M were 6.23 and 4.06 CFU/mL, respectively [45].

For pressure treatment (400 MPa/10 min, 500 MPa/5 min, and 600 MPa/2.5 min) low acid foods like sweet potatoes reduced Y&M to below detection levels where the initial count was 6.06 log10 CFU/mL. Further, Y&M was not detected for up to 84 days when samples were stored at 4 and 25°C. However, better quality was reported in samples stored at 4°C, indicating the importance of storage temperature [52]. Similarly, in cantaloupe puree, pressure treatment of 400–500 MPa for 5 min drastically reduced Y&M, and no regrowth was observed up to 10 days of storage at 4°C [31]. A comparative effect of sustained pressure treatment, pressure pulses, and pressure cycles was done on pineapple juice and nectar inoculated with *B. nivea*. It was concluded that at 600 MPa pressure, the effect of cycles was more effective in *B. nivea* ascospore inactivation than treatment under sustained high pressure. In addition to ascospores, Y&M counts were also reduced to below detection levels [55].

Y&M spores are readily inactivated at 400 MPa except for certain ascospores of heat-resistant mold such as *Byssochlamys nivea*, *Neosartorya fischeri,* and *Talaromyces macrosporus*.[56]. In general, these ascospores are often associated with spoilage of pasteurized fruit products also, such as juice, jams, purees, and candied fruits. Besides, their presence in processed food may cause deleterious effects due to the production of mycotoxins. Santos et al. [57] identified twelve highly resistant mold species, including *Neosartorya fumigate* (23.6%), *N. fischeri* (19.1%), and *Byssochlamys nivea* (5.5%) being the predominant species in high acid pasteurized fruit products such as strawberry puree, orange juice, and apple puree. The resistance of these ascospores depends on the spore age and species. The older the spore higher its resistance to processing.

HPP has been successfully applied for the effective inactivation of different pathogens in various fruits, vegetables, and their products. The amount of pressure and time required to inactivate the microorganisms depends on the food category. Optimized process parameters conditions for one product cannot be generalized for all the products. Among the different factors that plays important role in achieving microorganism inactivation are type and age. As bacteria, Y&M against pressure offers varying resistance. Combination treatment is reported to be more effective against spores.

2.2. Milk and Milk Products. Treating milk by high-pressure breaks only ionic and hydrophobic bonds of macromolecules (proteins) but does not denature bioactive proteins present in it. Very little or no effect on small molecules of milk components (vitamins, flavor, and amino acids) color, and other nutritional components have been reported along with effective microbial inactivation [58]. Other desirable changes induced are denaturation, gelling, and aggregation of proteins, which also influence the yield of dairy products produced from treated milk. Various researchers have successfully treated milk [59] and milk products like cheddar cheeses [60], gorgonzola cheese [61], and Queso Fresco cheese [62] using HPP for extended shelf life (Table 2).

Raw milk acts as a carrier for the transmission of bacteria like *E. coli*, *Salmonella*, shigella, and *S. aureus.* These microorganisms become part of untreated milk while milking milk from animals in barnyards, transporting milk, and storing milk at chilling centers. These food-borne pathogens are of public health concern. Yang et al. [66] worked on the inactivation of these bacteria in milk by HPP treatment. The duration of pressure treatment for 20, 30, 40, and 50 min at 300 MPa exhibited the highest inactivation rate of *Salmonella* and the lowest inactivation rate of *S. aureus*. The satisfactory duration for milk treatment was optimized to be 30 min. With an increase in pressure from 100 to 200 MPa, an increase in inactivation rates was observed. The inactivation was slower for *Salmonella* and *E. coli* and rapid for *Shigella* and *S. aureus*. It was concluded that a pressure of 300 MPa for 30 min at 25°C was sufficient to cause bacterial inactivation in milk. Most resistant *S. aureus* must be considered an indicator bacterium in milk when HPP was employed as a preservation technique. Efficacy of HPP in the destruction of *Mycobacterium avium* ssp. *Paratuberculosis* in milk was done by Donaghy et al. [64]. Pressure at 500 MPa for 10 min resulted in a 6.52 log reduction of the target microorganism.

A study was conducted by Narisawa et al. [72] to assess the injury and inactivation of *Escherichia coli* K-12 in different mediums, i.e., skimmed milk and its protein fractions (casein, whey, globulin, and albumin) by HPP treatment. It was revealed that skimmed milk had the most remarkable protective effect on inactivation. Moreover, the shielding effect was enhanced with an increase in the concentration of skimmed milk [72]. The presence of casein and lactose in milk also shields bacteria in milk during HPP [73]. The divalent cations Ca^{2+} and Mg^{2+} also shield bacteria against high-pressure-induced inactivation due to their stabilizing effect over the cellular membrane [16]. So, it is important to know the medium composition to optimize the pressure effective in overcoming the shielding effect provided to microorganisms by food.

Evidence of the repair mechanism of injured microbes in food, especially for low acid foods, has been reported, questioning the microbiological safety of foodstuffs.

Table 2: Effect of HPP to inactivate microorganisms in milk and milk products.

Product	Pathogens	Treatment (MPa/min/°C)	Log reduction*	Shelf-life (days/°C)	Optimum conditions (MPa/min/°C)	Reference
Milk	*L. monocytogenes*	400/0–25/20–25	5	—	400/4/20	Hayman et al. [63]
Milk	*Mycobacterium avium* ssp. *Paratuberculosis*	400–600/5–10/20	6.52	—	500/10/20	Donaghy et al. [64]
Milk	*S. aureus* ATCC 6538*E. coli* ATCC 25922*S. aureus* ATCC 25923*L. monocytogenes*ATCC 19115	400/21–31/0–50	6688	—	400/30/21–31	Viazis et al. [65]
Milk	*Salmonella, Staphylococcus aureus*	100–500/10–50/25	66	—	300/30/25	Yang et al. [66]
Cheese slurries	*Penicilliumroqueforti* IMI 297987, *E. coli* K-12	50–800/20/10–30	65	—	>600/20/20 or >400/20/30	O'Reilly et al. [67]
Cheddar cheese	*L. innocua*	200/five 1 min cycles/28	3–4	—	200/five 1 min cycles/28	Kheadr et al. [60]
Gorgonzola cheese	*L. monocytogenes*	400–700/1–15/—	5	—	600/10 or 700/5	Carminati et al. [61]
Soft-curd cheese	*S. aureus*	400/10/20	7	30/8	400/10/20	López-Pedemonte et al. [68]
Queso fresco	*L. monocytogenes*	200–600/5–20/20–40	5	84/4	600/5/20	Tomasula et al. [62]
Goat milk cheese	Mesophilic aerobic, *Enterobacteriaceae, Listeria* spp	400–600/7/10	1.61.11.5	60/4	600/7/10	Delgado et al. [69]
Yogurt	*Streptococcus thermophilus*	400–600/15/—	7	28/4	600/15/—	Jankowska et al. [70]
Whey-lime beverage	Mesophiles, yeast, coliforms	500/10/25	8	120/4	500/10/25	Bansal et al. [71]

*Log reductions are cited from the most lethal parameters after HPP treatment.

Mechanism of repair after injury of most pressure-resistant strains of two Gram-positive (*L. monocytogenes CA* and *S. aureus 485*) and Gram-negative bacteria (*E. coli* O157:H7 933 and *S. enteritidis FDA*) inoculated in milk, was studied [74]. Inoculated milk was given HPP treatment (350–550 MPa) and was stored at 4, 22, and 30°C. Three stages of microbes after pressure treatment were established: i.e., (i). Cells can form visible colonies plated in both selective and nonselective agar called active cells (AC), (ii). Cells that undergo structural injury like cell wall/cell membrane injury and can form colonies only on nonselective agar are called I1 injury or primary injury, (iii). Cells that undergo metabolic injury cannot form colonies in both selective and nonselective agar are called I2 injury or secondary injury. However, in the repair of I2 injury, cells can form colonies on nonselective agar, similar to I1 injury. Except for *L. monocytogenes CA,* other bacteria were inactivated or injured in milk at 350 MPa. *S. aureus* cells in milk after pressure treatment at 350 and 450 MPa observed on day 1 after storage at 22 and 30°C showed I1 type injury. Whereas pressure-treated milk samples at 350 MPa, after 1 day of storage, caused *E. coli* cells to repair from I1 state to active cell. Therefore, storage temperature and duration can alter the repair of bacterial cells, thereby influencing microbiological safety. Studies suggest that after the HPP of food, immediately injured cells might not be present but can recover during storage. So, a strategic combination with other processing techniques might effectively prevent the recovery of injured cells.

Yogurt is a fermented beverage prepared from milk in cooperation with two homofermentative bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*. The excess lactic acid bacteria in yogurt have attributed beneficial effects; nonetheless, the post-acidification during the cold chain and modification of viable lactic acid bacteria count are bottlenecks. Thermal processing after fermentation, in this case, would not be a viable solution to preserve the lactic acid bacteria at desired levels, and therefore novel technique like HPP plays a role. The effect of high-pressure treatment on the microflora of yogurt was investigated by Jankowska et al. [70]. They found that high-pressure treatment at 500 MPa does not significantly decrease the inactivation rate. In contrast, pressure treatment at 600 MPa/15 min showed a significant increase in bacterial inactivation from the initial load of ~10^8–10^9 CFU/mL to ~10^2–10^3 CFU/mL. It was found that *Streptococcus thermophiles* were slightly more resistant to high pressure than *L. bulgaricus*. Besides, yogurt was found to maintain acidity throughout the storage period after HPP treatment as it sufficiently reduced the acidifying bacteria. It was concluded that pressure treatment of 550 MPa for 15 min was optimum for yogurt processing with good sensory and textural characteristics with a shelf life of 4 weeks at 4°C. Microbial survivability in yogurt depended on the initial bacterial load and acidity of the sample [70]. The development of uniformly consistent microstructure in probiotic yogurt with improved gel strength and viscosity was accomplished by

treating milk with HPP before fermentation. The development of uniformly consistent microstructure in probiotic yogurt with improved gel strength and viscosity was accomplished by treating milk with HPP before fermentation [75].

Cheese is a fermented dairy product in wide demand all over the world. Improved characteristics of cheese were reported after HPP. Studies showed that high pressure imparts the following: (i) *e* alters the proteolytic activity of cheese [76], (ii) Improve the softness of cheese [77], (iii) Affect the rennet coagulation of milk [76], (iv) Increase the shelf life [76], (v). Increase the cheese yield [76], and (vi) Improve the physicochemical properties of soft cheese [78]. In corresponding to microbial inactivation of cheese under high pressure, several studies have shown promising results in improving shelf life without affecting its inherent quality. The effect of high-dynamic pressure on different types of milk and its effect on the quality of the cheese was studied by Kheadr et al. [60]. They found that 3-4 log reduction in *L. innocua* and 2–4 log reduction in total viable bacteria count was achieved by pressurizing milk, specifically the reduction in the microbial count was higher in low-fat milk. The reason is that milk fat acts as a protective medium for bacteria under high dynamic pressure, thereby preventing its destruction [60]. Thus, applying high-pressure to skim milk or low-fat milk employed for cheese preparation resulted in cheese being firm, cohesive, less brittle, and compact protein matrix with satisfactory microbiological quality. The cheeses prepared from low-fat pressurized milk show an initial listeria count of 10^6 CFU/mL was decreased to 10^2 CFU/mL after 3 months of ripening. Delgado et al. [69] reported that HPP at 400 and 600 MPa for 7 min of raw goat milk cheese resulted in inactivation of Mesophilic, aerobic, *Enterobacteriaceae,* Lactic acid bacteria, and *Listeria* spp., and differences in texture were observed. But the differences in control and pressure-treated samples were not observed by trained panelists and consumers. López-Pedemonte et al. [68] investigated the effect of ultrahigh pressure homogenization (UHPH) and HHP processing on the inactivation of *S. aureus* CECT 976 in milk to be employed for cheese making. The UHPH was employed at 300 and 30 MPa at primary and secondary homogenization stage, resp., followed by HHP treatment at 400 MPa/10 min/20°C. They found that *S. aureus* was present in cheese initially at a load of 8.5 log10 CFU/g in control. After UHPH and HHP treatment of milk, the cheese after 15 days of ripening showed complete inactivation of *S. aureus* and its enterotoxin.

In general, flavor, color, and nutrients were significantly retained after the pressure treatment of milk and its products. However, to prevent the recovery of injured cells during storage, a strategic combination of HPP with other thermal and nonthermal treatments can be looked upon.

2.3. Meat, Poultry, and Seafood. Meat, poultry, and seafood are high in moisture content and rich in protein and thus, these products have been associated with frequent outbreaks of food poisoning and food-borne diseases. Major outbreaks were linked with dog meat in China [79], red meat caused infectious intestinal infection disease outbreaks in the United Kingdom [80], and multiple outbreaks were due to frozen oyster in Australia [81]. A survey by FDA reported the presence of *L. monocytogenes* in cold-smoked salmon with a 17% frequency and 4% incidence in hot smoked fish and shellfish [82]. In Europe, 191 cases of death due to the eating of crustaceans and shellfish in which the presence of *Listeria* was reported in the year 2013 [83]. Listeriosis outbreak has been increasing in Europe for the last few years, with a fatality rate of 13.8% in 2017 EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) [84]. These implications could be minimized by using HPP, which also simultaneously retains the natural aroma, appearance, flavor, texture, and nutrient value of the products [85, 86].

The effect of high-pressure processing on deboned dry-cured hams was investigated by Perez-Baltar et al. [87]. They found that high pressure of 600 MPa for 5 min inactivated *L. monocytogenes* at the surface during 60 days of storage at 4 and 12°C. However, variation in the moisture content, water activity, and salt and nitrate content on the surface and interior of dry-cured ham showed variation in pathogen inactivation. Around 2 log reduction in surface and 3 log reduction in the interior of dry-cured hams were accomplished during HPP treatment. As per USDA and European criteria, food safety against *L. monocytogenes* could be attained by HPP treatment at 600 MPa/5 min. A study on the inactivation of Shiga Toxin-Producing *Escherichia coli* (STPE) by HPP treatment (400–600 MPa/0–18 min) was studied by Porto-Fett et al. [6]. Major conclusions drawn from the study were that refrigerated and frozen storage of meatballs prior to HPP resulted in similar pathogen reduction, i.e., 0.9 to 2.9 log CFU/g at 4°C and 1 to 3 log CFU/g −20°C. Only 1–3 min were required at 600 MPa as compared to 9 min at 400 MPa to achieve $a \geq 2.0$ log CFU/g. In another study, HPP treatment significantly reduced microbes in pork burgers [88]. The addition of 2% rice bran extract followed by HPP treatment at 600 MPa/5 min did not significantly affect microbial reduction but improved the quality of the pork burger. Rice bran extract acted as a natural antioxidant in maintaining the stability of burgers during refrigerated storage. Nevertheless, in comparison to rice bran extract, HPP treatment was effective in microbial inactivation and in extending the shelf life of pork burgers up to 21 days. Bonilauri et al. [89] reported the effect of the processing method and HPP on reducing *Salmonella* Spp. in Italian salami production. From the 20 different samples of salami, they identified a significant relationship between *salmonella* reduction and process parameters time/temperature of acidification/drying, time/temperature of seasoning, pH, and aw) during sausage production. The management of sausage production process parameters decreased the salmonella load by 0.97–4.67 Log CFU/g but was insufficient to achieve 5 log reduction requirements of export to the USA, whereas the additional hurdle in the form of HPP treatment resulted in 2.41–5.84 Log CFU/g. The study aimed to identify and implement appropriate HACCP plans to control the risk of *salmonella* in Italian Salami using the appropriate HPP technique.

Meat, poultry, and fish are perishable products with limited shelf life, and microorganisms easily thrive over them during various stages such as cutting, mincing, protein solubilizing with salt, product forming, or packaging [90]. HPP was used to inactivate the histamine-forming bacteria in tuna meat slurry, and HPP treatment resulted in morphological changes in the cells [91]. Direct HPP treatment caused morphological changes in the cell membrane, biochemical reactions, and the genetic mechanism of microorganisms, resulting in the microorganism inactivation. However, the effect is variable on microorganisms and depends upon different pressure and holding time. The total plate count and *Enterobacteriaceae* count of filleted tuna chunks decreased with an increase in pressure (100–300 MPa/5 min/25°C) compared to control tuna. The shelf life of filleted tuna chunks was increased up to 30 days at 2°C when packed in EVOH multilayered films after HPP treatment at 200 MPa [92]. However, in albacore tuna minced muscle, HPP treatments (275–310 MPa/2–6 min) resulted in a bacteriostatic effect on mesophiles and psychrophiles observed, i.e., were not able to kill microorganisms but were effective to prevent their proliferation during the shelf-life study. Treatment at 310 MPa for 6 min was most effective and improved the shelf life of minced albacore tuna for >22 days at 4°C and >93 days at −20°C. In addition, lipid stabilization, color, and texture improvement were also reported [93]. HPP can inactivate microorganisms and could be used for gelation without applying thermal treatment to achieve product characteristics close to fresh. HPP is currently employed in the USA by the oyster industry for shucking purposes, eliminating the need for costly skilled labor and reducing microbial risk to consumers by inactivating *Vibrio* spp. The oysters processed after HPP treatment in the USA (brand name plastic gold band) have received several national awards for quality products. Shelf-life of oysters was reported to be extended for 12 days at 4°C, and optimum conditions were found to be 300 MPa/2 min [94].

Several products such as minced mackerel [95], spreadable smoked salmon cream [96], raw chicken [90], pork paste [97], ready-to-eat meat [98], cold-smoked salmon [99], smoked salmon mince [100], oysters [101], albacore tuna minced muscle [93], black tiger shrimp [102, 103], and hilsa fillets [104] have been satisfactorily processed by HPP (Table 3).

Based on the reported literature on meat, poultry, and seafood, it can be concluded that HPP is a very effective technique for providing microbial safety and under optimized conditions HPP leads to an extension in shelf life by almost double or more. The pressure is also very effective in denaturing the proteins responsible for holding the meat within the shell of oysters, mussels, crabs, and shrimp. Therefore, a higher yield can be obtained as meat separation from the shell become more effortless and effective.

2.4. Other Products. HPP has also been utilized for products such as rice wine [121], ginger paste [122], maize [5], honey [123], and human milk [124]. Processing rice wine (non-heat pasteurized) at 392 MPa by Hara et al. [121] gave a shelf-stable product by inactivating Lactobacilli and yeast. The taste was equivalent to the untreated food sample. *Fusarium* mycotoxins, i.e., deoxynivalenol and zearalenone (produced by *F. graminearum)* were effectively decontaminated using HPP treatment (550 MPa/20 min/45°C) in maize [5].

A study on the effect of HPP on the reduction of native microflora of Mexican multifloral honey was reported by [123]. This study evidenced that high-pressure processing at 600 MPa for 12 min resulted in a reduction of 0.8 $\log_{10}$ of total mesophiles and 2.4 $\log_{10}$ of Y&M counts. A similar study by Akhmazillah et al. [125] on manuka honey has also reported that a high-pressure of more than 350 MPa at 40°C for 3 min reduced the bacterial load from 6 logN CFU/g to 3 logN CFU/g. Pressure-mediated treatment (600 MPa for 5 min) extended the shelf life of ginger paste for six months under refrigerated conditions. Both HPP and pasteurization were equally effective in reducing microbial population, but retention of bioactive components was more in HPP [122]. Rocha-Pimienta et al. [124] worked on the inactivation of *Bacillus cereus* and *S. aureus* vegetative cells in human milk by HPP. The pressure intensity and holding times needed for maximum inactivation up to 5.81 and 6.93 log CFU/mL were 593.96 MPa for 3.88 min. Waite et al. [126] indicated that the HPP of ranch dressing reduced the *Pediococcus acidilactici* by more than 6.4 log CFU/g. The studies carried out by eminent scientists proved that HPP is a viable process to improve the food safety of food products with extended shelf life.

In baked goods, cakes and batters were studied for their microbial, physical, and structural changes due to HPP [127]. The mesophilic aerobic bacteria Y&M were more susceptible to high pressure, causing a reduction from 4.3 to 3.8 log CFU/g and 1.7 to 1.0 log CFU/g, respectively, at 600 MPa for 6 min. The wheat dough also showed a similar reduction in the total aerobic mesophilic count, and Y&M count within 1 min of treatment at all pressures studied (50–250 MPa) [128].

Cereals and pulses being nonperishable commodities were not extensively studied for microbial aspects of high pressure. However, HPP was studied for its effect on starch modification, improving nutritional quality, water absorption, gelatinization, and development of quick-cooking rice [129–132]. Ravichandran et al. [133] investigated the effect of high pressure on water absorption and gelatinization of Paddy (*Basmati cv.*). Presoaked and unsoaked grains were pressure treated at 350, 450, and 550 MPa for a temperature of 30, 40, and 50°C for a duration of 300, 600, 900, and 1200 s. The highest moisture content of up to 50% (dB) was achieved at 550 MPa/50°C/1200 s in addition to 25% of gelatinization. Yu et al. [134] studied the effect of high pressure on cooked rice dominated with *Bacillus* spp. with *B. subtilis* and *B. cereus* population. They found that HPP treatment at 400 and 600 MPa increased the shelf life of cooked rice to 8 weeks at 25°C. HPP can be a very useful technique in reducing the microbial count of maize, honey, ranch dressing, and dough and in extending the shelf life of ginger paste and cooked rice.

Table 3: Effect of HPP to inactivate microorganisms in meat, poultry, and seafood.

Product	Microflora	Treatment (MPa/min/°C)	Log reduction	Shelf-life (days/°C)	Optimum conditions (MPa/min/°C)	Reference
Sliced vacuum-packaged dry-cured ham	*Salmonella enteritidis*	400–600/5/12	4.3	60/8	600/5/12	Alba et al. [105]
Poultry meat	MesophilesPsychrotrophs	60–450/15/20	3.2–3.85.2	—	450/15/20	Yuste et al. [106]
Bovine muscle	Total microflora	50–600/0–5/10	2.5	8/4	520/5/10	Jung et al. [107]
Raw marinated meats	Aerobic total count psychrotrophic bacteria, Yeast and *Enterobacteriaceae*	600/6/31	42	120/4	600/6/31	Garriga et al. [108]
Meat balls	Shiga toxin-producing*Escherichia coli*	400–600/0–18/—	<2		400/9/—600/3/	Porto-Fett et al. [6]
Low-fat pastramiStrassburg beefExport sausageCajun beef	Aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria* spp., *Staphylococci, Brochothrix thermosphacta*, coliforms, and fungi	600/3/20	4	98/4	600/3/20	Hayman et al. [98]
Smoked salmon mince	*L. innocua, Micrococcus luteus Pseudomonas fluorescens*	207/23/−20–25 (pressure shift freezing)	22.54.6	—	207/23/−20–25 with the release of pressure after 18 min	Picart et al. [100]
Oysters	*Cryptosporidium parvum*	305–550/0–360/—	6.5	—	550/3/—	Collins et al. [101]
Albacore tuna minced muscle	Total mesophiles and psychrophile	275–310/2–6/10	100–400 CFU/g	>22 days at 4°C and >93 days at −20°C.	310/6/10	Ramirez-Suarez and morrissey [93]
Oysters	Total aerobic count and anaerobic bacteria	260–600/5/20	2	31/2 on ice	400/5/20	Cruz-Romero et al. [109]
Minced trout	*L. innocua*	150–517/5/20	>4	—	414/5/20	Basaran-Akgul et al. [110]
Chicken breast fillet	*E. coli* KCTC *1682, S. typhimurium* KCTC 1925, *L. monocytogenes KCTC 3569*	300–600/5/15	6–8	7–14/4	600/5/15	Kruk et al. [111]
Yellowfin tuna chunks	Total plate count, *Enterobacteriaceae*	100–300/5/25	11	20/4	200/5/25	[92]
Dry cured ham	*Salmonella enteric*	347–852/2.3–15.75/7.6–24.4	4	—	525/15.5/16525/12/7.6600/12.1/16600/5/23.5	Bover-Cid et al. [112]
Dry cured hamDry cured ham	*EnterococcusfaecalisSerratia liquefaciens*	347–852/2.3–15.8/7.6–24.4347–852/2.3–15.8/7.6–24.4	46	—	750/9.5	Belletti et al. [113].Belletti et al. [114]
Black shrimp	*E. coli*, *S. aureus*	100–435/5/25	1.531.16	—	435/5/25	Kaur et al. [102]
Beef (frozen)	*E. coli* O157: H7	551/4/−35	1.4–1.7	—	551/4/−35	Lowder [115]
Chicken meat	*L. innocua*	200–400/5–15/0–40	8	—	400/10/0	Bulut et al. [116]
Chicken nuggets	*Enterobacteriaceae*	300/5/27	3	30/4	300/5/27	Devatkal et al. [117]
Smoked rainbow trout fillets, Fresh European catfish fillets	*L. monocytogenesE. coli*	200–600/1–5/—	>6	41/47/4	600/5/—	Mengden et al. [118]

Table 3: Continued.

Product	Microflora	Treatment (MPa/min/°C)	Log reduction	Shelf-life (days/°C)	Optimum conditions (MPa/min/°C)	Reference
Vacuum packaged mutton patties	Total plate count	200–400/10/—	2–3	—	400/10/—	Banerjee et al. [119]
Mussels	Total plate count	100–400/5/30	2	28/2	300/5/30	Bindu et al. [120]
Oysters	Aerobic plate count	100–300/1–3/20	1.27	12/4	300/2/20	Rong et al. [94]
Deboned dry-cured hams	*Listeriamonocytogenes*	400–600/5–10/—	3	60/4	600/5/—	Perez-Baltar et al. [87]
Pork burger	Total aerobic mesophilic bacteria and psychotropic bacteria	600/5/10	3	21/4	600/5/10	[88]
Italian salami	*Salmonella* spp.	600/—/14	>5		600/—/14	Bonilauri et al. [89]

ND = not detected; *log reductions are cited from most lethal parameters after HPP treatment.

3. Microbial Inactivation by HPP Assisted by Other Processing Techniques

HPP is an effective technique to inactivate or eliminate vegetative microorganisms but does not substantially affect spores [135]. pH in the case of fruits is low (<4.6) due to inherent acidity. It is further reduced by compression, so partially injured cells of microorganisms by HPP will not be able to recover in such a hostile environment. The difficulty is in the case of low acid products (poultry, meat, and milk) where pH values are >4.6. Spores grow even after HPP as soon as they find a suitable environment to grow and ultimately spoil the food [12]. To achieve higher efficiency for spore inactivation present in food samples by HPP alone, spores need to be germinated at low pressure in the first stage. Then in the second stage, pressure needs to be elevated to inactivate germinated spores. But using HPP twice over a product increases the processing time and energy consumed and, subsequently cost of the product. Moreover, a combination of pressure and temperature (which can be increased along the HPP) eliminates the step of the spore's growth by HPP [136]. So, using a combination mode (HPP and temperature simultaneously) helps achieve rapid heating and cooling of products, reducing processing time and product cost [135].

Combining HPP with other nonthermal (irradiation, ultrasound, and pulsed electric field) and mild heat techniques (pasteurization, blanching, and drying) will be an additional hurdle for the microorganisms. Also, beyond 600 MPa pressure, there is an exponential increase in equipment cost, and not considered economical. Some authors proposed the use of antimicrobial preservatives (nisin, chitosans, and pediocin) to achieve a synergistic effect with pressure and to reduce process severity [13, 137–140]). Microbial inactivation by HPP assisted by other processing/preservation techniques has been attempted by eminent researchers such as irradiation of chicken breast [141], irradiation of lamb meat [142], irradiation of kefir [14]; ultrasonication of *Rhodotorula rubra* [143, 144]; use of preservatives such as lysozyme, ethylene diamine tetraacetic acid (EDTA) [145], and nisin [146]. Hauben et al. [145] found that cells were more sensitive toward pressurization in the presence of preservatives. Effectiveness of hurdle technology consisting of HPP (400 MPa/30 min/70°C), pH (4), and nisin (0.8I U/mL) to completely eradicate spore of *Bacillus coagulans* (2.5 CFU/mL), whereas pressure alone (400 MPa) at ambient temperature and neutral pH had no significant effect on viable spores [146]. Paul et al. [142] showed that either irradiation (1.0 kGy) or HPP (200 MPa for 30 min) only reduced staphylococci ($10^4/g$) by 1 log cycle in lamb meat whereas, in combination, staphylococci can be completely eradicated. The complete deactivation of the microbial population (*lactobacilli, lactococci,* and yeast) of kefir was achieved using irradiation (5 kGy) and HPP (400 MPa/5 min/5°C) without changing structure and nutritional components (proteins and lipids) by Mainville et al. [14]. Treatment of *Bacillus subtilis* spores (400 MPa/30 min) and *E. coli* (300 MPa/10 min) using HPP followed by alternating current (50 Hz) leads to lethal damage to their cell component [147]. High pressure (500 kPa) in combination with heat (70°C) and ultrasound (117 db at 20 kHz) resulted in the inactivation of 99% of the *Bacillus subtilis* spore population [144]. Knorr [143] stated that HPP and ultrasonic individually were not adequate for inactivation of *Rhodotorula rubra* but complete inactivation was achieved in combination mode. The carbon dioxide-assisted HPP is one of the effective nonthermal technologies which has been applied successfully by different researchers for inactivating microorganisms and reported promising results [148]. This method utilizes moderate pressures (<50 MPa) sequentially or simultaneously with CO_2 to pasteurize liquid foods without compromising quality attributes. Pressure-ohmic-thermal sterilization is a novel technology involving the utilization of high-pressure in combination or consecutive application of ohmic heating for low acid foods to achieve a sterilization effect and simultaneously reduce the severity of the individual effect of temperature on quality attributes [149]. A study on ultrafiltration in combination with HPP

Table 4: Microbial inactivation by HPP assisted by other processing techniques.

Technique	Treatment conditions		Sample	Target Microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Irradiation	200/30/—	1 kGy	Lamb meat	*S. aureus*	The count of *staphylococci* was below detection level when both techniques were applied in combination where as individual treatment reduced the count only by 1 log cycle	Paul et al. [142]
	680/20/80	2 kGy	Chicken breast	*Clostridium sporogenes*	The dose required to achieve the eradication of *C. sporogenes* reduced from 4.1 to 2 kGy in combination with HPP	Crawford et al. [141]
Modified atmospheres	150/10/5	50% O_2 + 50% CO_2	Atlantic salmon	*L. monocytogenesS. typhimurium*	The combined application was found to be more effective in retaining microbiological characteristics with the extended life of 10 days at 5°C.	Amanatidou et al. [153]
HPCD	10.3/15/36	Supercritical CO_2	Beef trimmings	Total plate count, *E. coli* O157: H7, *E. coli, Salmonella* spp.	Log reductions of 0.83, 0.93, 1.0, and 1.06 were observed in total plate count, *E. coli* O157: H7, *E. coli* and *Salmonella* sp, respectively	Meurehg [154]
	14/40/45	Supercritical CO_2	Soy sauce pasteMarinated porkLoins	*E. coli, L. monocytogenes, S. typhimurium, E. coli* O157: H7	Percentage reduction of 33.81, 37.96, 37.48, and 36.84% was achieved in *E. coli, L. monocytogenes, S. typhimurium,* and *E. coli* O157: H7, respectively.	Choi et al. [155]
	12/30/35	Supercritical CO_2	Boneless pork loins	*E. coli, L. monocytogenes, S. typhimurium, E. coli* O157: H7	Log reductions of 1.5 1.4, 1.56, and 1.0 were achieved in *E. coli, L. monocytogenes, S. typhimurium,* and *E. coli* O157: H7, respectively.	Choi et al. [156]
	10/10/50	Supercritical CO_2	Pears	*S. cerevisiae*	4 log reduction was achieved	Valverde et al. [157]
	22–25/2–10/43–60	Supercritical CO_2	Apple juice	*Coliform,* total aerobic bacteria, Y&M	Complete inactivation of coliform, Y&M was achieved, and 3.72 log cycle reduction was achieved in total aerobic bacteria	Xu et al. [158]
Thermo-sonication and HPP as pretreatment	600/15/—	Acoustic energy density 20.2 W/mL	Orange juice	*Alicyclobacillus acidoterrestris*	HPP treatment with thermosonication was found to be most effective and the temperature used was 8°C lower than the temperature used in thermal treatment.	Evelyn and silva [151]
Heat Ultra-sonication Static pressure	300–500	Heat 70–90°C Ultrasonic 90–150 μm at 20 kHz	Buffer media	*B. subtilis*	Spores inactivation was observed to be highest at 70–90°C/300 kPa,/117 μm/20 kHz/6 min	Raso et al. [144]

Table 4: Continued.

Technique	Treatment conditions		Sample	Target Microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Ultrafiltration	500/6/—	Ceramic membrane (0.05 μm)	Apple juice	Total plate count and Y&M	For ultrafiltration + HTST and ultrafiltration + HPP, both treatments apple juices were microbiologically safe but higher retention of phenol and lower degree of browning was observed in the later treatment.	Zhao et al. [150]
Modified atmospheres packaging (MAP)	300/5/20	30%CO_2/70% N_2	Fresh chicken breast fillets	*Total viable counts, Pseudomonas, LAB, Brochothrix thermosphacta, coliforms, E.coli*	Combination treatment extended the shelf life up to 28 days	Rodrıguez-Calleja et al. [15]
Thermal pasteurization with Nisin	400/4/—500/2/—	Pasteurization at °C/15 s with nisin-100 IU/mL	Cucumber juice drinks	Total plate count and Y&M	Longer shelf life was achieved with 500/2 with nisin compared to other treatments.	Zhao et al. [159]
Nisin Heat	300–700/7.5–17.5/30–70	Nisin-0 to333 IU/mL.	UHT milk	*Clostridium botulinum spores*	To achieve 6 $\log_{10}$ cycle reduction best optimum conditions were 545 MPa/51°C/13.3 min and nisin at 129 IU/ml concentration.	Gao and ju [137]
Nisin; Ultrasound	300/3.3/5	1 mg/L34.6 W/30 s	Liquid whole egg	*L. seeligeri*	Nisin with HPP was more effective in reducing *Listeria seeligeri* (5 log reduction) as compared to HPP and ultrasound in combination.	Lee et al. [160]

Table 4: Continued.

Technique	Treatment conditions		Sample	Target Microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Heat	700/2 pulse/80	80°C	Tomato puree	*B. stearothermophilus*	High-pressure in combination with elevated temperatures resulted in an ambient-stable product, in which all the spores were inactivated.	Krebbers et al. [161]
	400–700/0–5.5/105	105°C	Egg patties	*B. stearothermophilus*	4 log reductions using pressure-heat treatment at 700 MPa/5/105°C min were achieved as compared to 1.5 log reduction in thermal processing 121°C/15 min.	Rajan et al. [162]
	800/-/60–80	60–80°C	Hamster brain homogenate	Prion	Infectious scrapie prions were effectively inactivated at 800 MPa (3 × 5 min cycles) at 60 and 80°C	Heindl et al. [163]
	700–900/0–32/80–100	80–100°C	Milk	*Clostridium* sporogenes spores	Spores were more sensitive to temperature as compared to pressure	Ramaswamy et al. [164]
	600/3/60–70	60–70°C	Tris buffer, skimmed milk, and orange juice.	*B. subtilis*	Slow compression and slow decompression were more effective than fast compression and fast decompression.	Syed et al. [165]
	600/-/75–105	75–105°C	Tomato juice	*B. coagulans*	Time taken to reduce the microbial counts were less using the combination treatment compared to individual treatment by thermal processing.	Daryaei and Balasubramaniam [166]
	200–350/0–2/105–150	105–150°C	Whole milk and phosphate-buffered saline	*B. amyloliquefaciens*	The temperature was the main driving force for inactivation and fat does not provide any shielding effect to microorganisms.	Dong et al. [167]
	300–900/1/60–80	60–80°C	Pumpkin puree	*Coliforms, Bacillus, E. coli, C. perfringens*	High-pressure-assisted thermal processing leads to a significant reduction in *Coliforms*, viable spores of *Bacillus* spp., mold and yeast. *E. coli* and *C. perfringens* were <1 log CFU g^{-1} (under limit)	García-Parra et al. [168]
	650/10/55–65	55–65°C	Soup	*B. subtilis*	Combined treatment reduced the *B. subtilis* by 4.5 logs. Also, this treatment took less time compared to static and agitating retort alone.	Ates et al. [169]
High pressure-assisted thermal processing	Step 1 = 100–200/7/—Step 2 = 586/10/23–77	80°C/10 min	Meat products	*C.perfringens*	The study purpose was activation of spores at low pressure (100–200 MPa/7 min) or elevated temperature (80°C/10 min); then germination at high temperature (80°C/10 min) followed by inactivation at high-pressure (586 MPa/23–73°C/10 min)	Akhtar et al. [170]

PEF: pulsed electric field; HPCD: high-pressure dense phase carbon dioxide.

Table 5: Microbial inactivation by HPP assisted by preservatives.

Technique	Treatment conditions		Sample	Target microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Bacteriocin (Lacticin 3147)	150–600/30/25	10000–15000 AU ml^{-1}	Cheese	*S. aureus, L. innocua*	The combined treatment resulted in substantial cell death (>6 log reduction), which exceeded that observed with either of these treatments alone.	Morgan et al. [171]
Enterocins A and B, Sakacin K, Pediocin AcH nisin	400/10/17	—	Meat model system	*E. coli, S. enterica, S. aureus, L. monocytogenes,Lactobacillus sakei, Leuconostoc carnosum*	At 4°C no of survivors remains the same as after combination treatment.	Garriga et al. [172]
Pediocin + nisin	345/5/60	Pediocin + nisin-5000 AU/g	Roast beef	*Clostridial species (C. sporogenes, C. perfringens, C. tertium,* and *C. laramie)*	Combination treatment increased the shelf life to 7 days at 25°C and 84 days at 4°C where as HPP alone has a shelf life of 42 days only at 4°C	Kalchayanand et al. [140]
Nisin	250–500/5/20	0, 250, 500 IU/ml	Milk	*E. coli*; *P. fluorescens; L. innocua; lactobacillus viridescens*	Combining high-pressure and nisin resulted in a greater inactivation of microflora (>6.4 log reduction) than when either was applied individually.	Black et al. [13]
Lysozyme	300/15/25	224 UA/ml	Skim milk and banana juice	Gram-negative bacteria including, *E. coli, Shigella flexneri, Yersinia enterocolitica,* and *S. typhimurium*	Combination treatment enhanced the inactivation of 3.6–6.5 log cycles and 0.5–2.1 log units in banana and milk, respectively.	Nakimbugwe et al. [173]
Edible film	300/20/15	The film contains oregano, rosemary, and chitosan.	Cold-smoked sardine	Total counts, sulfide-reducing bacteria	Synergistic effects were observed in combination plant-based extract reduced the total counts whereas film containing chitosan reduced both.	Gomez-Estaca et al. [174]
Organic acids	300–600/10/—	Potassium lactate-3% Mixture potassium + sodium lactate-3%, potassium lactate + sodium diacetate-2.5%	Spanish blood sausage (morcillas)	Total viable count, *Enterobacteria, Pseudomonas, Lactic acid bacteria, Clostridium perfringens*	15 days shelf life was achieved on the addition of potassium + sodium lactate followed by HPP as compared to other treatments.	Diez et al. [175]
Enterocins	400/10/17	Alginate films containing Enterocins 2000 AU/cm^2	Cooked ham	*L. monocytogenes*	A shelf life of 60 days was using combination treatment	Marcos et al. [176]

Table 5: Continued.

Technique	Treatment conditions		Sample	Target microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Enterocins A and B, sakacin K, nisin A, potassium lactate	400/10/17	Enterocins-200 or 2000 AU/cm^2, Sarkacin 200 AU/cm^2, nisin-200 AU/cm^2, potassium lactate-1.8%, and nisin (200 AU/g) plus potassium lactate (1.8%)	Sliced cooked ham	*Salmonella* spp.	A combination of HPP, antimicrobial packaging and refrigerated storage was an effective treatment to maintain a count <10 CFU/g.	Jofre et al. [139]
Nisin and potassium lactate	600/5/10	Nisin-800 AU/g, potassium lactate-1.8%, nisin (800 AU/g) plus potassium lctate (1.8%)	Sliced cooked ham	*Salmonella* spp. *L. monocytogenes* and *S. aureus*	A combination of antimicrobials with HPP treatment with refrigerated storage was effective in controlling growth up to three months but HPP was effective in reducing the growth of Salmonella spp. L. monocytogenes at 1 and 6°C for three months	Jofre et al. [139]
Enterocins A and B	400/10/17	Enterocins A and B-2000 AU/g	Fermented sausages	*S. enterica, L. monocytogenes,* and *S. aureus*	A combination of the ripening process, pressurization, and Enterocins was only effective in reducing the count of S. aureus.	Jofre, et al. [177]
Antimicrobial film	800/5/20	Films contained Carvacrol or allyl Isothiocyanate	Food model	*Botrytis cinerea* fungi	In combination lower intensity of both the treatment were utilized which would help develop low-cost technologies,	Raouche et al. [178]
Sodium lactate	600/2/20	2%	Cooked chicken	*L. monocytogenes*	After HPP treatment counts were below the detection limit but enhanced during storage within 21 days. Counts were below detection limit up to 105 days after combined treatment.	Patterson et al. [179]
Caprylic acid Purasal	110–700/10/5–40	Caprylic acid-0.15% Purasal (K-lactate + sodium diacetate)-2.5%	Cooked ham	*Carnobacterium divergens, Leuconostoc carnosum, Brochothrix thermosphacta,L. innocua and E. coli, O157:H7*	Carpylic or purasal addition in combination with HPP (600/10/10) enhanced the shelf life by 84 days	Vercammen et al. [18]
Mint essential oil	600/0–5/—	0.1 and 0.05%	Yogurt drink (ayran)	*L. monocytogenes and L. innocua*	To achieve the same level of inactivation combination treatment reduced the pressure by half (300 MPa) and time to 3.5 min	Evrendilek and balasubramaniam [180]

Table 5: Continued.

Technique	Treatment conditions		Sample	Target microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Nisin	600/5/—	Nisin directly-200 AU/cm^{2}Nisin through film-200 AU/cm^2	Ready-to-eat (RTE) sliced dry-cured ham	*L. monocytogenes*	Up to 60 days at 8°C HPP treatment with direct application of nisin was more effective.	Hereu et al. [181]
Nisin	100–500/30–60/50	0–1000 IU/mL	Apple juice	*Alicyclobacillus acidoterrestris spores*	HPP (200 MPa for 45 min) of apple juice containing nisin (250 IU/mL) resulted in more than 6 log reduction of Alicyclobacillus acidoterrestris spores	Sokołowska et al. [182]
Bacteriocins nisin, cinnamon, and clove		Nisin 500 IU/g, cinnamon oil-0.2% and clove oil-0.25%,	Rice pudim	*S. aureus*	Even mild treatment in combination with additives was effective in reducing the log cycles against S. aureus	Pulido et al. [183]
Enterocin LM-2 at 256 and 2560 AU/g.	200–400/10/—	256 and 2560 AU/g.	Refrigerated shelf life of sliced cooked ham	*L. monocytogenes, Salmonella, S. aureus psychrotrophic bacteria, aerobic* total plate count, *LAB, Enterobacteriaceae*	Enterocin addition (2560 AU/g), followed by HPP achieved >90 days shelf life.	Liu et al., [184]
Bacteriophages	200–600/5/10	1 : 1 of vB saus-phi-IPLA35 & vB saus-phi-IPLA88	Pasteurized whole milk	*S. aureus*	400 MPa was to be a promising treatment in combination with bacteriophages.	Tabla et al. [185]
Lactoperoxidase	250–450/10/—	Lactoperoxidase system	Smoked salmon	*L. monocytogenes*	Antimicrobial effects were observed after combination treatment but little alterations in quality attributes were also observed.	Montiel et al. [186]
Bovine lactoferrin	200–500/10/10	Bovine lactoferrin-0.5 mg/g.	Chicken filets	*E. coli O157: H7, Pseudomonas fluorescens*	Additional reduction of 2.3 log cycle was achieved in combined treatment of HPP (300 MPa) with bovine lactoferrin as compared to HPP alone	Del Olmo et al. [187]
Potassium lactate	600/6/10		Restructured hams	Aerobic mesophilic total counts, *Lactic acid bacteria, Enterobacteriaceae, L. monocytogenes, S. aureus, Salmonella spp.*	The addition of potassium lactate followed by HPP increased the reduction in the count.	Fulladosa et al. [188]

Table 5: Continued.

Technique	Treatment conditions		Sample	Target microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Potassium chloride + potassium lactate, sodium chloride	600/13/5		Smoked dry-cured ham	*L. monocytogenes Salmonella*	After pressurization (600/5 min) elimination of the pathogen took 14 days in ham containing sodium chloride whereas mix additives (potassium chloride + potassium lactate) took 28 and 56 days to eliminate Salmonella and Listeria, respectively. It indicates ham containing sodium chloride has more stability	Stollewerk et al. [189]
Nisin	600/5/15	Nisin	Dry cured ham	*L. monocytogenes*	During HPP treatment L. monocytogenes were found to be resistant but the assistance of HPP with nisin enhanced the inactivation	Hereu et al. [181]
Bacteriocins nisin and pediocin	400–500/10/12	Nisin-100 IU/g Pediocin-0.6%	Dry-cured ham	E. coli	Individual addition of nisin and Pediocin does not affect E. coli. But combined treatment HPP (500) and nisin maintained a synergistic effect up to 60 days	Alba et al. [190]
Enterococcus strains	600/5/15	Enterococci strain (*Enterococcus faecium* CTC8005, *Enterococcus devriesei* CTC8006 and *Enterococcus casseliflavus* CTC800) at ca. 3×10^6 cfu/g	Low-acid fermented sausages	*L. monocytogenes, S. aureus*	A combination of HPP and E. faecium CTC8005 was the most effective treatment. Individual addition of Enterococcus strains without HPP was not able to affect Enterococcus strains.	Rubio et al. [191]
Essential oils or their chemical constituents	175–400/5–20/—	200 μL/L	Orange and apple juices	*L. monocytogenes and E. coli*	Promising synergistic effects were achieved.	Espina et al. [192]
Sodium chloride, antimicrobial packaging	600/5/12	600/5/12 PVOH films containing nisin-450 AU/cm	Sliced fermented sausages	*L. monocytogenes*	No extra protection was achieved using the combination	Marcos et al. [193]
Chitosan based-coating containing nanoemulsion of Mandarin essential oil	200–400/5/25	—	Green bean	*L. innocua*	Synergistic effect of antimicrobials with HPP was more promising than with pulse light.	Donsì et al. [194]

Table 5: Continued.

Technique	Treatment conditions		Sample	Target microorganism	Observations	Reference
	HPP (MPa/min/°C)	Others				
Virulent bacteriophages	150–550/5–13	Phages (a cocktail of 3 *Shigella flexneri* or single *Vıbrio cholerae* phages, both applied at 109 PFU/mL)	*S. flexneri* in ground beef and *V. cholerae* in salmon and mussels	*S. flexneriV. cholerae*	To achieve an inactivation level similar to stand-alone treatment, combined treatment reduced the pressure and time there was more energy efficient pressure	Ahmadi et al. [195]
Marinating solutions (sodium chloride and citric acid)	300–600/5/—	1 or 2% for 18 hrs	Marinated beef	*L. innocuaEnterococcus faecium*	Combination treatment reduced both microorganisms by 6 log cycles and individual treatment with a marinating solution was not effective to reduce initial microbial count without HPP.	Rodrigues et al., [196]
HPP essential oils carvacrol and thymol, and thiol-reactive allyl-isothiocyanate and cinnamaldehyde	0.01–0.30%	450–600/3–15	Beef steaks	*L. monocytogenes* and enterohaemorrhagic *E. coli*	It was suggested by the author to use allyl-isothiocyanate and carvacrol practically to assist with HPP to achieve extended shelf life.	Li and Gänzle [197]

was conducted by Zhao et al. [150] and reported apple juice to be microbiologically safe with better quality attributes than UF + HTST (high-temperature short time) juice throughout the storage period of 60 days. Evelyn and Silva [151] used HPP as a pretreatment to enhance thermosonication effectiveness to eliminate *Alicyclobacillus acidoterrestris* spores in orange juice. To inactivate spores of pathogenic bacteria (*C. perfringens* and *B. cereus*) and spoilage microorganisms, i.e., bacteria (*Alicyclobacillus acidoterrestris)*, mold (*Byssochlamys nivea* and *Neosartorya fischeri*), and yeast (*S. cerevisiae)* present in food samples. HPP, thermal processing, high-pressure thermal processing, and thermal sonification was used. It was found that high-pressure thermal processing (600 MPa/20 min/70–75°C) was more effective in achieving reductions.

Moreover, a lower processing time was required to prepare a beef slurry, apple juice, strawberry puree, and beer [12]. Evelyn et al. [152] investigated the effect of high-pressure, high thermal treatments, and thermosonication treatments on the effect of *B. nivea* and *N. fischeri* mold spores. They identified that spores age has a profound effect on inactivation through HPP. For *B. nivea,* the reduction was 2.7 log for 4-week spores and 2 log for 12-week spores at 600 MPa/75°C/30 min. At the same treatment time, *N. fischeri* showed 2–4 log reduction, and 12-week-old spores were more resistant than 4-week-old spores indicating lower inactivation for older spores. On the other hand, thermosonic treatment at 0.33 W/mL at 75°C was not effective in the inactivation of ascospores. The high pressure of 600 MPa and temperature of 75°C would be appropriate while targeting the most resistant spores, i.e., old spores of >12 weeks.

Through combination treatment requirement of high temperature was reduced as required in individual thermal processing to achieve the same degree of inactivation with better quality and less energy. Similar results were found in the literature for using antimicrobial agents and preservatives. Treatments like ultrasonication and modified atmosphere packaging in combination with HPP were also found to provide a significant positive result in spores inactivation compared to individual treatment over food. Some of the literature describing the use of different techniques along with HPP is given in Tables 4 and 5.

4. Benefits of Technology and Engineering Challenges

Uniform and instantaneous pressure transmission are effective in causing the death of pathogenic microorganisms due to the permeabilization of cell membranes without much increase in product temperature. HPP can even be carried out at low temperatures. Cell membrane permeability changes are reversible at low pressure but irreversible at high-pressure. The effect of pressure occurs only on non-covalent bonds, and covalent bonds are not affected. Therefore, the characteristics of organoleptic and sensory properties remain unaltered, or the difference reported is not significant [16, 198]. Therefore, getting attention from the consumers and processors as the treated food is mildly processed and provides characteristics similar to fresh products. It is also effective in reducing enzyme activity, thereby enhancing the product's yield, quality, and shelf life, especially in fruits and vegetables [199]. Technology is environment friendly as no residues or waste are generated.

A variety of products can be treated using the technology, i.e., solid foods (preferably vacuum packaged) and liquid foods (in a flexible package, having the ability to bear compression up to 15 to 20%), dry-cured or cooked meat products, fish, seafood, marinated products, ready to eat meals, sauces, fruits and vegetables, juices, marmalades, jams, cheeses, milk, and other dairy products and nutraceutical [200, 201]. Some foods that cannot be treated by high pressure are: food packaged in glass since glass containers will break on compression; products like bread and mousse that have air included in them; spices and dry fruits as these products have low moisture content.

The equipment cost is high, and processed products have a niche market, so the product is commercially processed only in developed countries. It is due to the limited availability or development of large pressure vessels that can handle large volumes of food and withstand high pressures. Using one large pressure vessel rather than multiple small pressure vessels in parallel would be more effective and reduce operating and capital costs. The operating cost of the product is also dependent upon the operating parameters, i.e., amount of pressure, holding time, and temperature of the solvent used. Therefore, it is pertinent to optimize processing variables [16]. Challenges to the commercial application of high-pressure technology include material handling, process optimization, limited knowledge in understanding kinetic data, the role of constituents cleaning, and disinfection of equipment.

5. Conclusions

This review illustrates the effectiveness of the nonthermal technique, i.e., HPP, on microorganism reduction and extension of the shelf life of different food products. Food composition, type and age of microorganism, amount of pressure, and treatment time play an important role in reducing the microorganism load. This novel technology is very effective against vegetative pathogens but has some limitations in the inactivation of spores. Effective and synergistic results in the inactivation of spores can be obtained when combined with other thermal and nonthermal techniques. This combination of hurdles reduces the severity of individual processing while retaining the nutritional quality of food products. Although initial equipment cost is high, recent advancements and an increase in the number of HPP units have resulted in the successful commercialization of HPP products in developed countries and are also getting acceptance worldwide. Still, further work can be done to reduce the equipment cost and further research on the resistance of microorganisms.

Data Availability

All data pertaining to this review are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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