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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

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Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety

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Abstract

The last two decades saw a steady increase of high hydrostatic pressure (HHP) used for treatment of foods. Although the science of biomaterials exposed to high pressure started more than a century ago, there still seem to be a number of unanswered questions regarding safety of foods processed using HHP. This review gives an overview on historical development and fundamental aspects of HHP, as well as on potential risks associated with HHP food applications based on available literature. Beside the combination of pressure and temperature, as major factors impacting inactivation of vegetative bacterial cells, bacterial endospores, viruses, and parasites, factors, such as food matrix, water content, presence of dissolved substances, and pH value, also have significant influence on their inactivation by pressure. As a result, pressure treatment of foods should be considered for specific food groups and in accordance with their specific chemical and physical properties. The pressure necessary for inactivation of viruses is in many instances slightly lower than that for vegetative bacterial cells; however, data for food relevant human virus types are missing due to the lack of methods for determining their infectivity. Parasites can be inactivated by comparatively lower pressure than vegetative bacterial cells. The degrees to which chemical reactions progress under pressure treatments are different to those of conventional thermal processes, for example, HHP leads to lower amounts of acrylamide and furan. Additionally, the formation of new unknown or unexpected substances has not yet been observed. To date, no safety-relevant chemical changes have been described for foods treated by HHP. Based on existing sensitization to non-HHP-treated food, the allergenic potential of HHP-treated food is more likely to be equivalent to untreated food. Initial findings on changes in packaging materials under HHP have not yet been adequately supported by scientific data.

KEYWORDS
allergen, chemical food safety, HHP, high hydrostatic pressure, microbiology, packaging

1 | INTRODUCTION

High pressure (HP) (also high hydrostatic pressure [HHP] or high pressure processing [HPP]) is a technology that immerses a product under water, and exposes it to a hydrostatic pressure of several hundred megapascal in a HP vessel. The product is commonly packed in a high-pressure-suitable packaging and held under pressure for certain times, until decompression. HP treatment can take place between 100 and 1000 MPa, and at temperatures between 0 and 120°C. Pressure range from 100 to 800 MPa and temperatures ranging from 20 to 60°C have been mostly investigated. A few minutes of treatment time has been mostly reported in many studies, and times up to several hours have been explored in certain model studies. However, pressure in the range of 200 up to 600 MPa at ambient or chilled temperature is usually applied in commercial pasteurization-equivalent food applications, with holding time rarely longer than 5 min. As the industrial process is based on pressure and performed at ambient or lower temperature where thermal load is considered as marginal, in the literature, it is often claimed that HHP has a potential for food preservation and shelf-life extension, while maintaining certain quality attributes of treated food, similar to those of untreated products or improved attributes compared to their thermal counterparts. For inactivation of spores, application of HP in combination with high temperature (100–120°C) is needed.

In principle, HHP is suitable for most foods, provided that they have a sufficient water content and no air voids. The main foods treated using HP today are meat products, fruit and vegetable products, aquatic products, and beverages (Table 1) (Huang et al., 2017).

In 2019, more than 550 commercial HP machines for food processing were in operation worldwide, 59% of them in North America, 24% in Europe, and 18% in Asia. This accounts for a global vessel volume of around 130,000 L and more than 1.5 Mil tons of foods processed annually (Tonello-Samson et al., 2020, 2018a, 2018b; González-Angulo et al. 2021). In addition, 25 commercial size machines were installed in research centers, universities, and similar centers for research and development. Around 65 units are owned by juice producers and around 25 machines operate in toll processing (machine owners offering their HHP services to other food companies) (Tonello-Samson, 2015, Personal communication, Hiperbaric, Spain; Tonello-Samson et al., 2020).

Compared to thermal or other nonthermal preservation technologies, HHP is considered as relatively expensive technology; therefore, it is particularly applied for high-quality foods with the aim of maintaining their fresh and nutritional character, similar to one of an untreated product. Treatment costs for HHP are usually between 0.1 and 0.3 EUR/kg, depending on the size of the HP vessel, filling ratio, and treatment conditions (pressure and treatment time). In the toll processing, often costs of around 0.45 EUR/kg are calculated, depending on the amount of product and achievable filling ratio. For a comparison, costs of a thermal treatment (e.g., high temperature short time, HTST) are usually around 0.005–0.01 EUR/kg, depending on the level of energy recovery, and costs of a treatment by pulsed electric fields (PEF) are around 0.01–0.2 EUR/kg, depending on throughput and annual operating hours (Aganovic, 2017; Heinz & Buckow, 2009).

HP is applied to food products with the following goals:

1. Inactivation of microorganisms, such as bacteria, viruses, and parasites of human health concern,
2. Shelf-life extension (inactivation of organisms and partial inactivation of enzymes),

TABLE 1 Examples of high pressure-treated foods on the market

Product	Process parameters	Purpose of treatment	Market share
Juices and beverages	400–600 MPa1–5 min	Microbial safety and extension of shelf-life	30%
Meat products (ham, cured meat, andcold cuts)	400–600 MPa1–5 min	Microbial safety	30%
Fruit and vegetable preparations (e.g., guacamole, baby food, dips, and salsa)	500–600 MPa5–10 min	Shelf-life extension and inactivation of enzyme (color preservation)	28%
Seafood and shellfish	250–350 MPa30–90 s	Removal of shell, increasing yield, inactivation of *Vibrio*	7%
Ready-to-eat meals	400–600 MPa1–5 min	Microbial safety and extension of shelf-life	3%
Dairy products	400–600 MPa1–5 min	Microbial safety and extension of shelf-life	2%

Source: Adapted from Hayashi (2002); Tonello-Samson (2018a).

3. Physical and chemical modification of food matrix (e.g., cold cooking)

Although the science of HP treatment of biomaterials began more than a century ago, and HHP has now been described and recognized as a technology with the potential to provide safe foods with high quality, there are still a number of remaining questions regarding the safe use of HP. Although HHP has been described and recognized as a technology with a potential to provide safe food with high quality, there are still certain risks associated with the application of the technology that need to be considered. Specifically, depending on the processing parameters (pressure–temperature–time) being used for treatment of food, potential risks that can arise include:

1. Survival of pathogenic microorganisms,
2. Survival of quality-related microorganisms (spoilage organisms),
3. Undesired (bio-)chemical reactions,
4. Undesired effects on increasing allergenicity.

The goal of this review, based on the available literature, is to identify potential effects of HHP on microorganisms and food structure and composition, with an emphasis on safety-related aspects, in combination and/or comparison to conventional, in particular thermal processes. Technical, packaging-related, legal, and environmental aspects of HP technology are considered in this review, and potential risks associated with the use of HHP for food are outlined. The review focuses on hydrostatic pressure only, which is at present the most common way to use HP. Other processes that use dynamic pressure, like homogenization, shock waves, or applications of compressed fluids, like treatments with supercritical CO_2 or supercritical water, are not addressed in this review.

2 | BRIEF HISTORICAL BACKGROUND OF HHP APPLICATIONS TO FOODS

Pioneering work on the application of HP was done in the United States by Bert Hite in 1899, reporting the preservation of milk that was "kept sweet for longer" when HP of 650 MPa for 10 min at room temperature was applied (Hite, 1899). In a follow-up report, it was suggested that HP can be used for the shelf-life extension of fruits and fruit products, but vegetables were considered "hopeless," due to the presence of spore forming bacteria (Hite, 1914). Several years later, Cruess (1924) suggested HP for the preservation of juices with low pH, conditions where the growth of spores is inhibited. In 1946, the American physicist Percy Williams Bridgman received the Nobel Prize in physics, stating "a fact of possible interest," as follows: "If the white of an egg is subjected to hydrostatic pressure at room temperature, it becomes coagulated, presenting the appearance much like that of a hard-boiled egg." However, public interest at that time was focused on shifts of the melting point of ice in relation to pressure (HPBB, 2016). Basset et al. report the effects of pressure on enzymes, viruses, phages, and bacteria (Basset et al., 1938; Basset & Macheboeuf, 1933; Basset et al., 1933). Investigations of the effects of pressure on the physico-chemical properties of food biopolymers were made in the 1960s by Payens and Heremans (1969), who described the effects of pressure on β-casein molecules in milk, and by Macfarlane (1973) who reported meat tenderization using pressure (Hendrickx & Knorr, 2001; Patterson et al., 2007).

The first HP-treated products, such as yogurts, jams, and jellies, were launched on the Japanese market in 1980. Around the same time, research on the application of HP for food processing intensified. As a result of significant research done over the ensuing years, more pressure-treated products were introduced into the market in the 1990s, such as guacamole in the U.S. market and sliced cooked ham in Spain (Balasubramaniam, 2016). The last two decades have seen a steady increase of HP technology used in the treatments of different foods. Accordingly, several manufacturers of HPP equipment that produce industrial-scale machines, such as Hiperbaric (Burgos, Spain), Avure (Middletown, OH, USA), Uhde (Hagen, Germany, merged with Multivac in 2011), Kobelco (Kobe, Japan), Baotou Kefa High Pressure Technology Co., Ltd. (Baotou, China), and several other smaller companies, have emerged that produce lab- to pilot-scale equipment. In total, more than 10 companies are manufacturing HPP equipment (Balasubramaniam et al., 2016). In 2013, the HPP equipment market was estimated at $350 Mil, and is projected to grow at a CAGR of 11.26% from 2016, to reach USD 500.3 Mil by 2022 (Marketsandmarkets, 2016).

3 | TECHNICAL ASPECTS OF HHP

To understand HHP and its effects on food and microorganisms, the fundamentals of HP, technical requirements for the equipment, progress in their development, and important process parameters are first reviewed.

3.1 | Fundamentals of HHP

By nature, pressure is an intrinsic property of a thermodynamic system and cannot be treated separately from other parameters like temperature, specific volume, or energy. This is expressed by Equation (1), where change in systemt's energy, expressed as Gibbs Free Energy (G), equals volume change with compression (or

decompression) subtracted by entropy (S) change with heating (or cooling) (Hawley, 1978).

$$d\ (\Delta G) = \Delta V dp - \Delta S dT \quad (1)$$

Equation (1) applies to systems that consist of one single substance or of multiple substances. From this basic energy balance, material properties of a system can be derived as functions of temperature and pressure. In biomaterials, this is often caused by structural alterations of molecular structure during the exposure to changes in the energetic status of the system. Therefore, transitions from a molecular phase A to a phase B occur at well-defined combinations of pressure (p) and temperature (T), and can be plotted as phase transition lines in a p-T diagram. The slope is given by the ratio of the difference in enthalpy (ΔH) and specific volume (ΔV):

$$\frac{dp}{dT} = \frac{\Delta H}{T \Delta V} \quad (2)$$

Since most foods are mixtures of numerous substances often present in different states (solid, liquid, or gaseous), direct applicability of Equations (1) and (2) is very limited, mostly due to the unknown functional relationships of changes in ΔV, ΔS, and ΔH, with p and T.

In a first approach, water, as the main constituent of many foods, can be used to identify the pressure and temperature domains relevant for food processing. The phase diagram of water shows that the range of 0–100°C, in which water is in a liquid state at ambient conditions, can be extended by an increase in pressure. While a shift of few 10°C is sufficient to freeze or evaporate liquid water, a pressure of 4 orders of magnitude is required for crystallization of water at 20°C. This phenomenon is not observed naturally on earth's surface and requires technical compression equipment, which was not introduced for scientific use before the end of the 19th century (Bridgman, 1912).

Under pressure, water exhibits unique phase change behavior between the liquid and solid state, and even the solid state is characterized by 19 different crystalline modifications (Loerting et al. 2020). The fact that the freezing point of water is reduced to approx. –20°C, when compressed to 200 MPa before it starts increasing with pressure, was prerequisite for the development of life on this planet. At 900 MPa, water solidifies at +20°C.

Biochemical, as well as organic or inorganic reactions, are influenced by pressure (and temperature). Pressure shifts reactions in equilibrium to the side of a reduced volume. Ionic dissociation reactions often behave in this manner and are well described by Equation (3), using the equilibrium constant K and the reaction volume change ΔV^*. A negative ΔV^* is indicating a shift toward the products (Knorr et al., 2006).

$$\frac{dK}{dp} = -\frac{\Delta V^*}{R\,T} \quad (3)$$

Pressure, at which the equilibrium is reached, can also have an effect on the rate k, which is described by Equation (4) using the activation volume $\Delta V^{\#}$ (Van Eldik et al., 1989):

$$\frac{dk}{dp} = -\frac{\Delta V^{\#}}{R\,T} \quad (4)$$

Although the thermodynamically derived Equations (1–4) form a theoretical background of the behavior of a biological matter under pressure, the evaluation of HHP for food processing is largely driven by empirical data. Studies often use idealized or simplified food matrices and its cohabitating microbiota, in order to obtain experimental results that can be generalized. The dilemma is, however, by doing so, the practical relevance with regard to food safety is often a constraint. A number of more recent review papers tried to come to a more general conclusion, but the vast amount of published results is sometimes difficult to compare, since the used experimental setups are not always comparable (Balasubramaniam et al., 2015; Knorr et al., 2006; Rastogi et al., 2007; Wang et al., 2016).

HP involves the principles of Le Chatelier, isostatic pressure, and microscopic ordering. Le Chatelier's principle describes that chemical reactions, conformational changes, or phase transitions in a system at equilibrium shift to the side leading to a volumetric reduction when applying pressure, while those which lead to a volume increase are inhibited. The isostatic pressure principle states that the pressure is transmitted instantaneously and uniformly in all directions of the sample independently of the sample's size and geometry. The microscopic ordering principle implies that the degree of molecular ordering of a substance increases, when pressure increases at a constant temperature (Elamin et al., 2015; Jaeger et al., 2012).

3.2 | Essential technical prerequisites

3.2.1 | Pressure vessel

Typical HP equipment comprises a cylindrical pressure vessel, HP generation system, yoke, process control to monitor temperature and pressure, and a material handling system (Elamin et al., 2015; Rastogi, 2013; Ting, 2010). In order to maintain the pressure and ensure pressure stability, different pressure vessels have been developed, from a single piece to more complex ones.

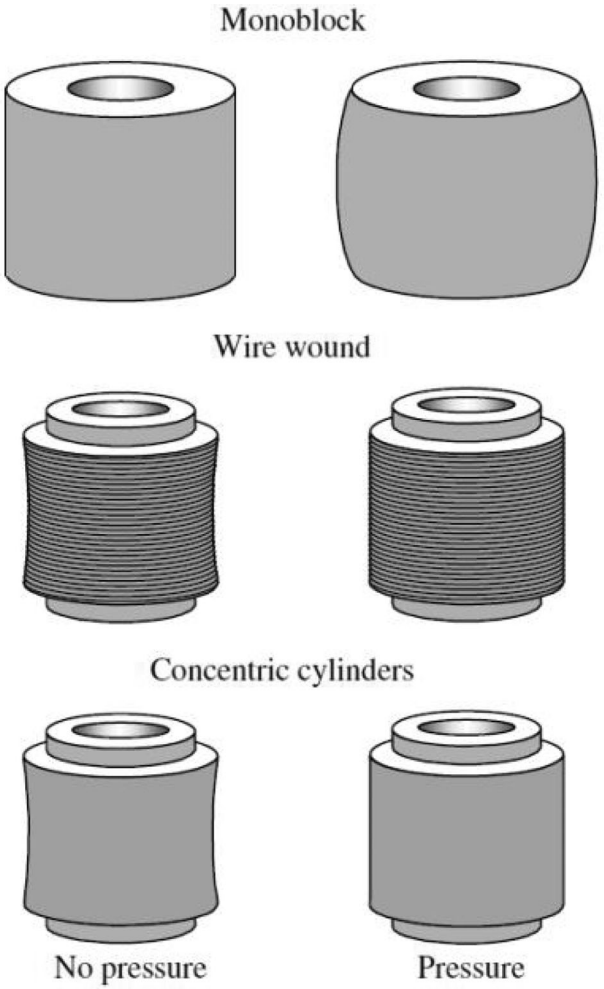

FIGURE 1 Different high pressure vessel designs––monolithic cylinder, wire wound, and concentric cylinders (Torres & Velázquez, 2008)

First pressure cylinders were simply monolithic cylinders made from low alloy steels. Its wall thickness is dependent on the vessel's inner diameter, maximum working pressure, and the number of cycles to be performed (Mertens, 1995). Thinner walls were realized by using wire wound, multilayer or other prestressed designs (Rao et al., 2014), as illustrated in Figure 1.

There are three different main techniques to design prestressed cylindrical vessels, namely (1) autofrettage, (2) heat-shrink, and (3) wire wound technique (Koutchma, 2014; Mathur, 2009), which are well described in more detail elsewhere (Koutchma, 2014; Moss & Basic, 2013; Sedighi & Jabbari, 2013).

Vessels constructed using wire wound technique have lifetimes of more than 100,000 cycles at pressures of 680 MPa or higher (Koutchma, 2014), today increasing to 200,000 cycles (Tonello-Samson et al., 2020).

Systems for HHP processing vary greatly in their size. Vessel volumes for commercial application range from 35 to 687 L. The vessel's orientation can be either horizontal or vertical. Horizontal cylinders allow the loading of the vessel from one side and unloading from the opposing side enabling single-direction product flows, whereas vertical designs need to be handled from the top (Koutchma, 2014). Vessels produced with heat-shrinking are characterized by increased lifetime, durability, maximum applicable pressure, and a reduced weight of the shell (Elamin et al., 2015). Larger vessel sizes with consistent mechanical properties are usually constructed by using wire-winding technology, whereas small sizes are realized using monolithic metal alloy cylinders, which are less cost intensive.

3.2.2 | Pressure transmitting medium

In the pressure vessel, usually a fluid (often water) surrounds the products and acts as the pressurizing medium. Alternatively, fluids other than water can be used; however, factors, such as corrosion prevention properties, fluid viscosity changes under pressure, heat of compression, and effects on foods, would have to be taken into consideration. The medium transmits the pressure to food products equally from all sides, thus preventing foods from crushing during HHP (Rastogi, 2013). Due to the surrounding fluid, products need to be packed prior to processing. In contrast, a liquid food may act itself as pressure transmitting medium if being pressurized. The treated liquid food can be directly filled or transferred to reservoir tanks (Elamin et al., 2015).

3.2.3 | Pressure generation systems

Two different techniques to build up the pressure in the system can be generally applied–direct (Figure 2––left) and indirect (Figure 2––right) pressure generation. HP can be directly generated using a moving piston, which varies the specific volume inside the pressure vessel. Thereby, the pressure can be increased or decreased depending on the piston's position. In contrast, indirect compression varies the quantity of pressure fluid to adjust the pressure in the vessel. Therefore, a reservoir tank with pressure fluid is connected via pressure tubes to the vessel. A system consisting of a pump and valves adjusts the quantity of pressure fluid in the pressure vessel. To generate HHP, fluid is pumped from the reservoir tank into the vessel increasing the pressure applied, whereas fluid is pumped from the pressure vessel into the reservoir tank to decrease the pressure.

A major drawback of direct compression is the need to ensure the integrity of the seal between piston and vessel during HPP. Consequently, in industrial HPP systems, mainly indirect pressure generation systems are applied.

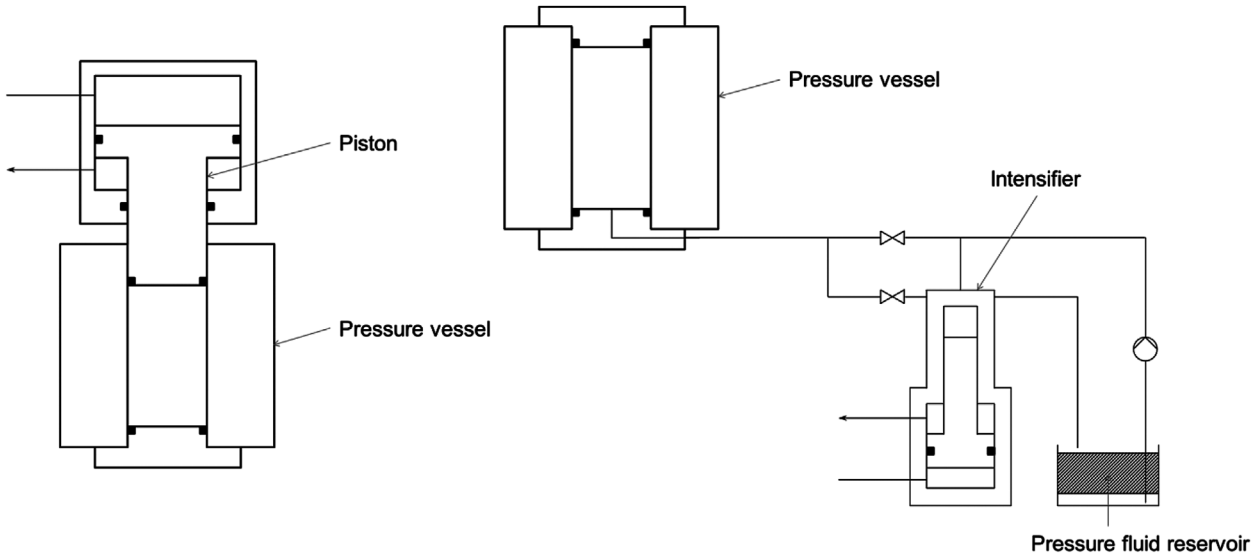

FIGURE 2 Graphical illustration of direct (left) and indirect (right) pressure generation adapted from Mertens (1995)

3.2.4 | Mode of HP operation

Industrial applications of HHP for food products are realized either as batch or semicontinuous operation modes. Solid and liquid food products, also with large solid particles, can be processed in batch mode, whereas liquid and pumpable food products, such as juices and milk, can also be treated in semicontinuous systems (Balasubramaniam et al., 2015; Ting & Marshall, 2002).

In batch processing, food products are first filled into flexible packages, sealed and then either placed directly in the pressure chamber or in a handling system, that transfers the products into the pressure vessel. After loading the product in the pressure vessel, isostatic pressure is built up by either indirect or direct means of pressure generation. After reaching the desired pressure, the pressure is held for a predetermined time. The pressure vessels are discharged by either transferring the pressure transmitting medium to the reservoir tank or moving a piston out of the vessel (Elamin et al., 2015). Finally, the products are removed from the vessel, dried, and stored until final distribution (Rao et al., 2014).

In semicontinuous systems, fluids and pumpable products can be directly pumped in and out of the pressure chamber through HP tubes, valves, and isolators. After HPP, the treated product is filled aseptically in packages (Rao et al., 2014). Semicontinuous systems have two or more vessels containing a free floating compression piston. When one vessel is unloaded from the product, a second is compressed. Optionally, a third vessel is loaded with the product at the same time. Thus, a nearly continuous product flow may be realized (Balasubramaniam et al., 2015). In 2019, the French company Ateliers Hermes Boissons was the first one investing in the HHP system, capable of producing juices before bottling and allowing for a capacity of around 4000 L/h (Tonello-Samson et al., 2020).

Dynamic HP homogenization is a continuous processing operation that applies HPs to liquid products. This paper focuses on static HPP of food products, the application of dynamic HP is not further discussed herein.

3.3 | Process parameters

During HHP, food products are typically exposed to pressures of around 200–600 MPa. Industrial applications aim for short cycle times of less than 5 min to maximize throughput, reduce costs, and increase commercial competitiveness (Koutchma, 2014). This typically allows for five to six cycles in an hour (Elamin et al., 2015), depending on the pressure come-up time (CUT), and time required for loading and unloading the product.

Depending on the products' composition and properties, at a pressure of 600 MPa, products are compressed by up to 15% (Mújica-Paz et al., 2011). Due to the compressive work against intermolecular forces, the temperature of the product and the pressure-transmitting media increases with increasing pressure. This phenomenon is known as adiabatic heating (Knoerzer et al., 2010). The temperature change upon pressure change can be described according to Equation (5):

$$\frac{dT}{dP} = \frac{T\alpha_p}{\rho C_p}, \tag{5}$$

where dT is the temperature change, dP is the pressure change, T is the initial temperature, α_p is the volumetric expansion coefficient, ρ is the density, and C_p is the isobaric heat capacity of the material (Juliano et al., 2009). The temperature change for most of the food products with a high moisture content is similar to that of the water (~3°C per 100 MPa). Nevertheless, it depends on product properties (Elamin et al., 2015; Gupta & Balasubramaniam, 2012). In heterogeneous products, areas with different adiabatic heating might occur, since the compressibility may be different for different foods and food constituents (Mor-Mur & Saldo, 2012). In systems where a pressure-transmitting medium is pumped into the vessel to pressurize the system, a temperature gradient may occur when the temperature of the incoming liquid differs from the liquid in the vessel (Abdul Ghani & Farid, 2007a,b; de Heij et al., 2003). Temperature gradients during processing may lead to a variability in inactivation kinetics for enzymes and microorganisms (Hartmann & Delgado, 2002, 2003). In this respect, approaches for improving temperature uniformity have been investigated, for example, to use carrier systems in the pressure vessel with an insulating effect (Knoerzer et al., 2010; Knoerzer, Juliano et al., 2007) or integration of a heating system for preheating polymeric liners for carrying the samples (Juliano et al., 2009). Setting the temperature higher than the target initial temperature in the pressure vessel reduces heat loss to the vessel walls. Recirculation of water prior to pressure build-up enables reaching of initial target temperature of compression fluid, sample carrier, and samples after closing the chamber (Juliano et al., 2009). Temperature differences of up to 20°C are theoretically possible, especially at elevated treatment temperatures, and a complete temperature balance under these processing conditions is not possible. Treatment temperature below 20°C can only be maintained by applying external cooling of the HP vessel. Several research groups investigated modeling of the hydrodynamic and thermal changes during HHP (Abdul Ghani & Farid, 2007a; Abdul Ghani & Farid, 2007b; Hartmann, 2002; Hartmann & Delgado, 2003; Hartmann et al., 2003; Otero et al., 2007; Otero & Sanz, 2003). However, a lack of research on temperature variations under pressure has been identified (Mor-Mur & Saldo, 2012).

It is important to mention that possible temperature fluctuations during HHP are of less importance for the pasteurization equivalent than for the sterilization equivalent. The difference between HP pasteurization and sterilization equivalent depends on the temperature conditions applied during HPP. While for pasteurization equivalent pressures of 400–600 MPa are applied at ambient or chilled temperatures, to achieve sterilization equivalent, temperatures exceeding approximately 90–120°C upon pressurizing to 500–600 MPa are required (Balasubramaniam et al., 2015; Mújica-Paz et al., 2011; Nguyen & Balasubramaniam, 2011). These combined pressure-heat treatments are often referred as high pressure temperature (HPT) process, high pressure thermal sterilization (HPTS), or high pressure high temperature (HPHT). Figure 3 shows the temperature and pressure, as a function of the process time for the HP pasteurization and HP sterilization equivalent.

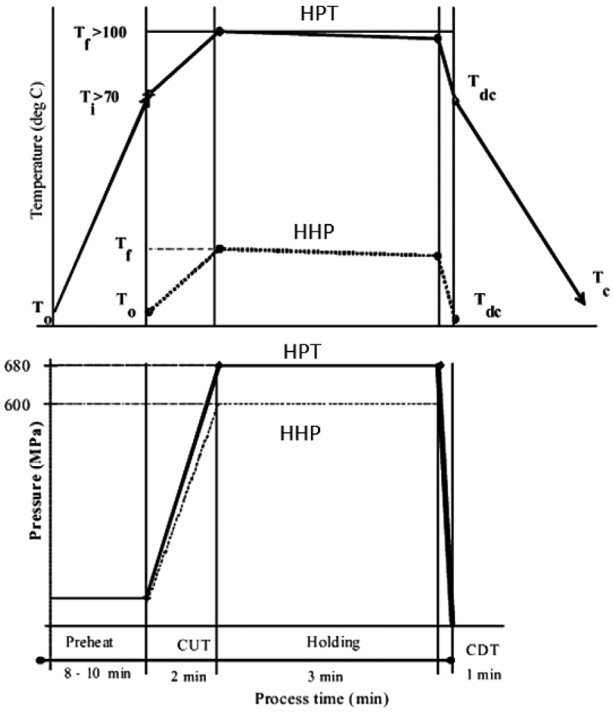

FIGURE 3 Temperature and pressure during a processing cycle for high pressure pasteurization equivalent (HHP) and high pressure sterilization equivalent (HPT) (adapted from Koutchma, 2014). The pressure come-up time (CUT) is defined as the time required for compression from atmospheric pressure to the target pressure. The pressure come-down time (CDT) is defined as the time required for decompression

During HPT process, the product in the pressure vessel is preheated to an initial temperature T_i. When the initial temperature is reached, the pressure in the vessel is increased to the target process pressure. The duration until compressing to the target pressure is identified as the pressure CUT, and it depends on product compression rate and pressure-transmitting media. Furthermore, the CUT is proportional to the power of the pressure pump, size of pressure intensifier, restrictions of HP tubing and valve loss, and the target process pressure. After reaching the target pressure, the system holds it for a selected time. In the last step, the HP vessel is decompressed to the atmospheric pressure and the time required for the decompression is identified as the pressure come-down time. It is worth noting that application of temperatures above 100°C and pressure above 400 MPa can cause material problems of the HP

vessel. A pressure level of 1000 MPa is currently considered as a technical maximum limit of industrial HP treatment.

4 | MICROBIAL SAFETY ASPECTS OF HHP

The complexity and variability of food-specific properties, such as pH value, a_w value, contents of salt, sucrose, or other ionic/nonionic substances, texture, and other attributes, make it difficult to make a general statement on microbial safety of HP processed food. In the following, HP inactivation of vegetative bacteria (equivalent to thermal pasteurization, typically resulting in 5–7 log reduction of microbial population) and bacterial spores (equivalent to thermal sterilization, 12 log reduction in microbial population) is discussed. More detailed information on inactivation of bacteria by HHP in different food matrices is provided in Table 3, and inactivation of spores in Table 4. The inactivation of human pathogen viruses and parasites that can be transmitted by food is also reviewed, and more detailed information is provided in Table 5 and Table 6, respectively. Tables 3 to 9 can be found in the suplementary material.

4.1 | Inactivation of vegetative cells by HP (pasteurization equivalent)

Vegetative cells of food-associated microorganisms are inactivated at ambient temperatures with HHP in the range of 200 up to 600 MPa, as usually applied in commercial food applications (Georget et al., 2015; Mota et al., 2013; Rastogi et al., 2007; Wang et al., 2016). Numerous studies are available describing killing curves for vegetative cells of pathogens and/or spoilage organisms in different foods or model systems thereof. Mostly, the kinetics of inactivation by HHP is characterized by a continuously declining curve, in some instances followed by tailing for longer treatment times, a phenomenon known from thermal inactivation processes. For HHP treatments, tailing is caused by the genetic heterogeneity of microbial populations or occurrence of barotolerant cells (Mota et al., 2013), due to stress adaptation and selection (Tay et al., 2003). The effectiveness of HHP treatments for microbial inactivation has been frequently demonstrated in different food matrices, although the mechanisms of inactivation by HHP have been addressed in only a few studies. At low-pressure levels from 20 to 180 MPa, mainly sublethal cellular damages are occurring, whereas at levels from 200 to 400 MPa, lethal damages are contributing to the microbial inactivation of a large variety of food-associated microorganisms (Lado & Yousef, 2002; Serment-Moreno et al., 2014). Owing to the huge diversity among microorganisms in nature, there are marked differences in sensitivity and/or tolerance of microbial taxonomic units against HHP. In general, eukaryotic cells like yeasts and molds are more pressure sensitive than prokaryotic cells like bacteria, among which Gram positives are more tolerant than Gram negatives (Considine et al., 2008; Dumay et al., 2010; Georget et al., 2015). Many particular effects of HHP on vegetative microbial cells have been described, which are simultaneously acting and finally contributing to the cell death. In vegetative cells, not only the cell structural organization, but also its metabolic processes are affected by HHP. HHP of more than 300 MPa can lead to the unfolding and denaturation of proteins, which can also result in enzyme inactivation (Knorr et al. 2011). At sufficiently high pressure levels, phase transitions and changes of fluidity of microbial cell membranes are observed, leading to ruptures in the cell membrane and promoting denaturation of membrane proteins (Winter & Jeworrek, 2009). Moreover, disintegration of ribosomes in their subunits (Niven et al., 1999) and intracellular pH changes are discussed to be the major pressure-induced effects (Kaletunç et al., 2004; Molina-Gutierrez et al., 2002; Rastogi et al., 2007). It can be concluded from the literature that the inactivation of vegetative cells by HHP is a complex event, which depends on the interaction of numerous particular effects, finally leading to the cell death.

The efficiency of inactivation of vegetative cells in a certain food strongly depends on endogenic and exogenic factors prevailing during HHP treatment. The latter are mainly represented by the pressure and temperature, and numerous data are available in the literature describing the relation between these two factors. Interestingly, efficient inactivation of vegetative cells in food matrices can be also achieved at temperatures below ambient temperature (Arroyo et al., 1999), down to subzero temperatures (Realini et al., 2011; Ritz et al., 2008). The impact of individual endogenous factors may vary greatly; the overall effect may result from their possible combination and may strongly depend on the type of food matrix (Georget et al., 2015). The pH value, water activity, and/or concentration of ionic (e.g., sodium chloride) and nonionic (e.g., sucrose) solutes, as well as the entrapment of bacterial cells in components of food matrix, are discussed to have major effects. In general, the pressure-induced inactivation of vegetative cells is accelerated at low pH values, compared to neutral pH value. Taking the history of food and conditions of processing into account, this has a favorable effect on the production of safe HHP food with pH values below 4.5 (Molina-Gutierrez et al., 2002; Wouters et al., 1998). In the presence of solutes in foods and/or in foods with low water activity, the efficiency of inactivation can be impaired to varying extent. However, there is no clear

correlation between water activity and efficiency of HHP inactivation, suggesting that the individual solutes themselves may possibly trigger effects that are not due to an impact of water activity (Georget et al., 2015). A similar impairment of inactivation efficiency under HHP can also be observed in dehydrated food matrices (e.g., dried spices, milk powder, and dry-cured meat products) (Georget et al., 2015). Finally, any protection of the vegetative cells by food matrix components has to be taken into consideration, for example, entrapment of cells in fat particles or in the oil phase of emulsions, both representing special cases of low water activity components (Georget et al., 2015). Due to the various factors limiting the efficiency of HHP inactivation of vegetative cells in foods, new strategies have been developed to improve the microbial inactivation efficiency. The addition of natural antimicrobials like bacteriocins (e.g., nisin), and essential oils or active substances thereof (e.g., carvacrol and citral), has found to be promising to enhance the safety of HHP foods (Chien et al., 2017; Hauben et al., 1996; Ogihara et al., 2009). Interestingly, synergistic effects between the HHP-induced microbial inactivation and the antimicrobial activity of natural antimicrobials have been described (Ogihara et al., 2009).

In addition, microbial susceptibility to HHP depends on the history of cells in the food matrix, affecting the physiological status of the cell. Besides the growth status (e.g., exponential and stationary), expressed stress responses caused by intrinsic and extrinsic factors might impair the efficiency of inactivation, for example, heat or cold shock, osmotic or acidic stress (Rendueles et al., 2011; Duru et al., 2020). As shown for *Listeria monocytogenes*, bacterial cells entering a dormant, long-term-survival phase can become more barotolerant (Wen et al., 2009). More recently, genomic analysis of the barotolerant *L. monocytogenes* RO15 and reference strains indicated that prophages and inhibited prophage defense systems may be involved in the barotolerance (Duru et al., 2020). Moreover, the strategy of using not only one but more HHP treatments could be more effective in controlling microbial and quality deteriorations (Zhang et al., 2015).

Mathematical models have been described to predict the inactivation of microorganisms by HHP as a function of the processing time. In general, a nonlinear behavior is typically observed in the pressure inactivation of microorganisms, which is supported by the findings that the microbial inactivation is a multifactorial event. In the past, numerous primary models being linear, concave, or sigmoidal have been developed to describe the inactivation kinetics by HHP (Klotz et al., 2007; Serment-Moreno et al., 2014). Models referring to mostly single target bacteria or yeasts, but also to groups of microorganisms, for example, aerobic bacteria, have been published not only for laboratory media (Cook, 2003; Koseki & Yamamoto, 2007), but also for numerous foods (Chakraborty et al., 2015; Parish, 1998; Pilavtepe-Çelik et al., 2009; Van Opstal et al., 2005). However, these models are applicable only, if the processing conditions like, for example, pressure, temperature, pH, are kept constant. For description of the effect of pressure and/or temperature on the predicted primary model parameters, nonlinear secondary models, for example, the Bigelow model (Santillana Farakos & Zwietering, 2011), have been developed and shown to be applicable for foods as well (Dogan & Erkmen, 2004; Koo et al., 2006; Pilavtepe-Çelik et al., 2009; Van Opstal et al., 2005). Up to now, no generic model for description of the microbial inactivation kinetics by HHP could be developed. The insufficiency of good-quality experimental data and the complexity of inactivation kinetics are discussed to be the reason for this gap (Serment-Moreno et al., 2014; Serment-Moreno et al., 2015). Thus, due to differences in the nature of target microorganisms, the diversity of microbiota in the different food products, as well as the complexity of food matrices, existing models have to either be adapted to the single case, or new models have to be developed to predict the microbial inactivation kinetics in the particular cases.

4.2 | Inactivation of bacterial endospores (sterilization equivalent) by HP

4.2.1 | Bacterial endospores

Bacterial endospores display a considerably higher resistance to HP than vegetative cells. Bacteria like *Clostridium* (*C.*) and *Bacillus* (*B.*) species are key for safety and spoilage of low acid (heat treated) preserved foods. Spores from such species can tolerate pressure treatments above 1000 MPa at room temperature. By combined pressure/temperature treatments, an inactivation of such food-relevant bacterial endospores is possible (Online Appendix Table 4). The heat resistance of various bacterial endospores does not correlate with high resistance to pressure (Margosch et al., 2004; Margosch et al., 2004; Olivier et al., 2011), but, in principle, the required inactivation temperature and/or time is lowered/shortened under pressure (Heinz & Knorr, 1996; Margosch et al., 2003; Reddy et al., 1999; San Martin et al., 2002; Wuytack et al., 1998).

4.2.2 | HPT processes for endospore inactivation

Specific HPT processes have been developed, which make use of the adiabatic heating during the pressure ramp, and aim at the sterilization of foods. Although the

inactivation of spores by heat and HHP has been studied for several decades, and knowledge of the underlying mechanisms has increased significantly, still not all aspects are well understood. Comprehensive overviews are available on pressure-based strategies for the inactivation of spores (Lenz & Vogel, 2015) and the behavior of endospores in complex food matrices (Georget et al., 2015).

Most of the studies on endospore inactivation under HHP conditions were conducted using model organisms, that is, mainly *B. subtilis* and other food-spoiling *Bacillus* species, for example, *Geobacillus stearothermophilus*, *B. coagulans*, and *B. amyloliquefaciens*. These studies deliver exemplary insight into the behavior of spores in food and molecular mechanisms of inactivation by HHP, including changes in the spore membrane, release of dipicolinic acid (DPA), and the involvement of the germination machinery, summarized by Reineke et al. (Reineke et al., 2013; Reineke et al., 2013). This insight is related to food spoilage organisms, that is, to the producers' risk of restricted shelf-life and commercial loss. However, the consumers safety, discussed here, exclusively derives from toxin-forming *Clostridium* and *Bacillus* species, for example, *C. perfringens*, *B. cereus* and, specifically, neurotoxin-forming *C. botulinum* types for which literature is rather scarce.

The differentiation between clostridia and bacilli is crucial, because the inactivation of bacterial endospores by pressure is generally considered to rely on pressure-induced spore germination, followed by inactivation of germinated spores (Margosch et al., 2004; Reineke et al., 2013).

Available data for *B. subtilis* (Reineke et al., 2013), *B. amyloliquefaciens* (Margosch et al., 2006; Margosch et al., 2004), and *C. botulinum* (Lenz et al., 2015; Margosch et al., 2006; Margosch et al., 2004) suggest that there might be some basic commonalities between *Clostridium* and *Bacillus* spores including that (1) the ability to retain DPA rather than its amount present in the spore core is important for HPT resistance, (2) the activity of cortex lytic enzymes (CLEs) is likely not to be required for a rapid, nonphysiological DPA release, and (3) specific ranges of p/T parameter combinations, where a characteristic inactivation pathway is favored, overlap.

However, different shapes of isoeffect curves for DPA release and spore inactivation reflect large differences in the response of *Bacillus* and *Clostridium* spores to HPT treatments, for example, *B. subtilis* (Reineke et al., 2012; Reineke et al., 2013; Reineke et al., 2013), *B. amyloliquefaciens* (Margosch et al., 2006; Margosch et al., 2004), and *C. botulinum* (Lenz et al., 2015; Margosch et al., 2006; Margosch et al., 2004). Especially the efficiency of low pressure/moderate temperature treatments to induce DPA release via a physiologic-like germination pathway (Paidhungat & Setlow, 2000, 2001; Rode & Foster, 1966; G. Wang et al., 2011), as a first step toward inactivation, appears to be markedly lower from various *Clostridium* spores (Hölters et al., 1999; Lenz et al., 2015; Margosch, 2004; Reddy et al., 1999) than from *B. subtilis* spores (Doona et al., 2014; Georget et al., 2014; Gould & Sale, 1970; Kong et al., 2014; Margosch et al., 2006; Paidhungat et al., 2002; Reineke et al., 2012; Reineke et al., 2013; Reineke et al., 2013; Torres & Velazquez, 2005; Wuytack et al., 1998). Considerable differences in DPA release kinetics and inactivation rates can also be found at p/T conditions, where a nonphysiologic DPA-release is leading to rapid spore inactivation, that is, at elevated pressure levels and temperatures (e.g., *C. botulinum* [Lenz et al., 2015; Margosch, 2004; Margosch et al., 2004; Reddy et al., 1999; Reddy et al., 2006]; *B. subtilis* [Margosch, 2004; Reineke et al., 2013]).

These differences are likely to be related to differences between *Bacillus* and *Clostridium* spores concerning spore components involved in their HPT-mediated inactivation. Such differences can be found in nutrient germinant receptors, the signal transduction pathway during the early steps of germination and inner membrane properties/proteins (Paredes-Sabja et al., 2011). Another striking difference between *Bacillus* and *Clostridium* spores can be found in the CLE machinery they utilize. In addition to CLEs, which might be not essential for germination (e.g., SleM in *B. weihenstephanensis* and some *Clostridium* species), *Bacillales* species commonly rely on the YpeB–SleB–CwlJ–GerQ (YSCQ) pathway, with the CLE, SleB requires YpeBm (Burns et al., 2010; Cartman & Minton, 2010; Paredes-Sabja et al., 2011), and the CLE CwlJ (dependent on GerQ), while the Csp–SleC (CS) system with the CLE SleC (Gutelius et al., 2014), which requires activation by a Csp protease (Adams et al., 2013; Paredes-Sabja et al., 2009), is absent (Paredes-Sabja et al., 2011). *Clostridiales* can possess genes coding for either one or both pathways, where the majority of organisms are likely to rely on the CS system due to nonfunctional YSCQ (Paredes-Sabja et al., 2011). Nonphysiological DPA release was reported to be not limited by the activity of the two CLEs conserved among *Bacillus* species (Paredes-Sabja et al., 2011). However, the fact that CwlJ can be activated by DPA release (or exogenous Ca–DPA) and SleB has been suggested to be activated by cortex deformation (due to core rehydration), makes it likely that cortex degradation by such enzymes facilitates further DPA release and core rehydration during nonphysiological germination (Black et al., 2007; Paidhungat et al., 2002; Reineke et al., 2011; Reineke et al., 2013; Setlow, 2003; Wuytack et al., 1998). SleC is thought to be not activated in response to DPA release or core rehydration (Paredes-Sabja et al., 2008; G. Wang et al., 2012), which makes a similar reenforcing function unlikely. Thus, the sequential steps of a rapid DPA release, partial core

rehydration, CLE activation, cortex lysis, further core rehydration, and inactivation, proposed for *Bacillus* spores treated at HP/elevated temperatures, are unlikely to similarly take place in *Clostridium* spores. Even within the heterogeneous species of *C. botulinum*, it seems impossible to predict the effect of HPT treatments on spores, which becomes strikingly evident when looking at p/T zones of spore stabilization detected for proteolytic (Margosch et al., 2006; Margosch et al., 2004), but not for nonproteolytic (Lenz et al., 2015) *C. botulinum* strains.

In recent studies with proteolytic (types B) and nonproteolytic (type E) *C. botulinum* strains in food model systems and different types of real foods, spore stabilization by HHP against high temperature inactivation could be confirmed, namely for type B strains. It was demonstrated for *C. botulinum* type B that, at a given temperature (100, 110, and 120°C), spore inactivation was increased when pressure was reduced, for example, from 600 to 300 MPa (Maier et al., 2018).

Such fundamental differences given, it is unlikely that true surrogates for *Clostridium* spores for the purpose of evaluating the effectiveness of HPT processes can be found within *Bacillus* species. For the proteolytic *C. botulinum* types A and B, *C. sporogenes* could have the potential to function as a surrogate, because the difference between these organisms is limited to toxin formation. For the nonproteolytic type E, no surrogates can be suggested at the time being.

4.2.3 | Factors influencing inactivation of endospores by HP

Potential baroprotective or synergistic effects of endogenous food parameters, such as fats, sugars, salts, pH, and water activity on the HPT inactivation of bacterial endospores, are not well studied yet. In analogy to observations with vegetative cells, it has been speculated that solutes could also penetrate the inner spore membrane, interact with cell components and, possibly, lead to retarded DPA release and inactivation (Ananta et al., 2001; Georget et al., 2015; Sevenich et al., 2013; Sevenich et al., 2015; Sevenich et al., 2014). In a recent study, the HPT inactivation of *C. botulinum* spores in four specific ready-to-eat meals and model systems with identical pH values, water activity, and amounts of major food components (one substitute component for the major classes, fat, protein, carbohydrates, and salt) was compared. Although the shapes of the inactivation curves were similar for spores in model systems and real foods, the total numbers of survivors varied. Additionally, it was impossible to infer the effect of a single major food component. Matrix effects appeared to be dependent on the *C. botulinum* type, as spores isolated from fish tended to be protected from pressure by fish matrices, and meat isolates by matrices containing meat (Maier et al., 2017).

This suggests that the impact of food matrices on HPT endospore inactivation may be different from that reported for vegetative cells, and more complex and differentiated than previously suspected (Maier et al., 2017). The establishment of a generic approach for the evaluation of the impact of food components on endospore inactivation, to date, appears not to be possible.

Taken together, HPT inactivation of bacilli and clostridia deserves a differentiated consideration. For the determination of food safety with respect to toxin-forming clostridia, a case-by-case study is required for HPT-treated foods without any further hurdles following known principles of spore germination and growth inhibition by low temperature and/or pH value.

4.3 | Inactivation of viruses

The main viruses relevant for foodborne transmission are norovirus and rotavirus, which cause gastroenteritis, as well as hepatitis A and E virus, which cause infectious hepatitis. All of these viruses are nonenveloped RNA viruses, which generally show a high stability against most environmental conditions. As for human norovirus and hepatitis E virus, no efficient cell culture systems for determination of infectivity exist so far. Efforts to estimate their stability have mainly been done using closely related surrogate viruses. However, the distinct stability against a given environmental factor can vary remarkably between the viruses, but also between different types of the same virus species. This has been repeatedly shown in heat stability studies with viruses (Arthur & Gibson, 2015), and also by their HHP treatment, as reviewed below. In addition, food matrices may play an important role in virus stability and the commonly identified food matrices involved in disease outbreaks are different for the distinct viruses. Norovirus, rotavirus, and hepatitis A virus are shed via human feces and mainly contaminate food surfaces during handling of food. In addition, shellfish and berries have often been identified as virus transmission vehicles, which are contaminated through contact with sewage or wastewater during their growth. In contrast, hepatitis E virus is zoonotic and widely distributed in subclinically infected pigs and wild boars. Meat and meat products produced from infected animals may therefore serve as a source for human infection with this virus (Giannini et al., 2018; Szabo et al., 2015).

The distinct mechanisms of virus inactivation by HHP are not completely known so far. A destruction or deformation of the viral capsid was demonstrated after HHP,

which eliminated virus infectivity, whereas the antigenicity of the virus was largely maintained (Dumard et al., 2013; Lou et al., 2011). In contrast, the virus genome was obviously not, or only slightly damaged after HHP treatment (Lou et al., 2011; Sánchez et al., 2011).

4.3.1 | Inactivation of viruses by HP

Studies on the use of HHP for inactivation of viruses have been published for different virus species (Online Appendix Table 5). This includes hepatitis A virus (Pavoni et al., 2015), human norovirus (Li et al., 2013), avian influenza virus (Isbarn et al., 2007), rotavirus (Araud et al., 2015), human adenovirus (Kovac et al., 2012), as well as different human pathogenic picornaviruses (Kingsley et al., 2004), including the poliovirus (Kingsley et al., 2002). The majority of investigations focused on the inactivation of viruses in food intended for human consumption. However, HHP was also applied in order to develop inactivating procedures for vaccine production (Dumard et al., 2013), or to inactivate viruses in order to control the spread of animal-pathogenic viruses by imported meat products (Buckow et al., 2017). In most of the studies, a treatment at 400 MPa for 5 min at 4°C turned out to be effective for virus inactivation (> 4 $\log_{10}$ decrease). However, the efficiency of HHP was strongly dependent on several factors and especially on the investigated virus species leading to much higher pressure/time combinations necessary for a significant inactivation in many instances. In addition, different genotypes of the same virus species can show different stabilities during HHP, as shown for human norovirus (Li et al., 2013; Lou et al., 2016; Ye et al., 2015) and rotavirus (Araud et al., 2015).

4.3.2 | Factors influencing inactivation of viruses by HP

The efficiency of HHP is dependent not only on the magnitude and duration of pressure treatment, but also on other factors like temperature, pH value, salt concentration, and on the composition of the embedding matrix. Whereas several studies show a higher efficiency of HHP at low temperatures with an optimum at 0°C for norovirus (Huang et al., 2014; Lou et al., 2015), for hepatitis A virus in cell culture supernatant, better results were derived at 20°C as compared to 5°C (Kingsley & Chen, 2009). The effect of the pH value seems to be highly dependent on the distinct virus species. Whereas low pH values led to a better inactivation of hepatitis A virus (Kingsley & Chen, 2009), the inactivation was more efficient at neutral pH compared to low pH value for human norovirus (Li et al., 2013; Lou et al., 2016). Increasing concentrations of sodium chloride lead to a lower efficiency of HHP for hepatitis A virus (Kingsley & Chen, 2009). Also, the addition of calcium chloride showed a significant protective effect against HHP inactivation of norovirus (Sánchez et al., 2011) and adenovirus (Kovac et al., 2012). Hepatitis A virus was more efficiently inactivated by HHP in marinated shellfish as compared to nonmarinated shellfish (Pavoni et al., 2015). Norovirus was markedly more resistant against HHP inactivation on dried berries as compared to fresh berries (Huang et al., 2014; Li et al., 2013). A recent study confirmed differences in norovirus HHP stability when present in green onions as compared to salsa (Sido et al., 2017). Whereas most of the studies investigated only a few distinct pressure/temperature/time combinations, some efforts on the development of predictive models have also been made, which enable the prediction of virus reduction in a range of different parameter combinations (Buckow et al., 2008).

4.3.3 | Surrogate viruses and alternative methods for assessment of virus inactivation

Because no efficient cell culture system exists for human norovirus so far, most of the HHP studies for this virus were performed either by using surrogate viruses or molecular capsid integrity assays. For hepatitis E virus, study using feline calicivirus and bacteriophage phiX174 as surrogate viruses has been published (Emmoth et al., 2017). Mainly used surrogates for human norovirus were murine norovirus (Huang et al., 2014; Lou et al., 2011; Sánchez et al., 2011) or feline calicivirus (Chen et al., 2005; Kingsley & Chen, 2008). A recent study used Tulane virus, a monkey calicivirus, as a surrogate for human norovirus (Li et al., 2017). However, significance of the generated data with the surrogate viruses for the prediction of human norovirus behavior under the same conditions is a matter of debate (Richards, 2012). Until now, only one study has been published, in which the infectivity of human norovirus in oysters after HHP was directly measured through the ingestion by volunteers, followed by the analysis of their virus excretion (Leon et al., 2011). In this study, a treatment at 600 MPa for 5 min was necessary for complete inactivation of human norovirus. In contrast, murine norovirus was already completely inactivated after HHP at 400 MPa for 2 min, as assessed in a previous study (Lou et al., 2011). Also, the use of capsid integrity assays, in which intact virus particles are purified by binding to receptor molecules followed by detection of the packaged virus genome by RT-PCR, has to be interpreted with care. In most cases, no proof of these methods by direct comparison with infectivity assays has been presented. However, at least one study showed a reliable correlation of this

technique with an animal infectivity assay, in which gnotobiotic piglets were inoculated with human noroviruses, followed by measurement of virus excretion (Lou et al., 2015).

In recent years, significant progress has been made in the development of laboratory methods for infectivity assessment of human norovirus and hepatitis E virus. Recently published novel cell culture systems for human norovirus (Jones, Watanabe et al. 2014, Ettayebi, Crawford et al. 2016) are quite promising, but still very sophisticated and therefore not applicable to larger stability studies at the moment. In case of hepatitis E virus, cell culture-isolated strains from chronically infected patients seem to be promising for use in virus inactivation studies (Cook, D'Agostino et al. 2017).

4.4 | Food relevant parasitic protozoa and parasitic helminths

Parasites that can be transmitted via food and cause diseases in humans belong to protozoa or helminths. Protozoa are unicellular eukaryotes, which may be free-living or parasitic. Parasitic protozoa are able to reproduce in humans, which contributes to their spread, and can also lead to development of severe infections, even from a single organism. Protozoa, which are infectious to humans, include *Toxoplasma*, *Amoeba*, *Cryptosporidia*, *Sarcosporidia*, and flagellates, such as *Giardia* and *Leishmania*. Helminths are worms, which may also be free-living or parasitic. The best known parasitic representatives are tapeworms (*Cestoda*) and flukes (*Trematoda*) from the phylum of flatworms (*Platyhelminthes*), large roundworms (e.g., *Ascaris*), *Trichinella*, and *Anisakidae* from the phylum of roundworms (nematodes), and various human intestinal parasites, such as *Moniliformis*, from the phylum of thorny-headed worms (Acanthocephala).

Food-borne parasite infections are rare, but if occurring, they can lead to significant health implications. Among the 24 (potentially) foodborne parasites listed by FAO/WHO (2014) for risk classification, 14 can be transmitted through food of animal origin (marine as well as freshwater fish, freshwater crustaceans, pork, beef, wildlife meat and, more rarely, shellfish and milk). Food of nonanimal origin can be fecally contaminated and become a carrier of parasites (Painter et al., 2013).

Consumption of raw, contaminated, or spoiled products, as well as inadequate processing of products of animal origin, are the main causes of parasite transmission by food. Fish products, such as sushi, sashimi, and ceviche, are often infected with *Anisakis simplex* (Mo et al., 2014; Robertson, 2018), or rare cooked or uncooked meat products, such as tartar, carpaccio, and khao soi, are food examples where parasite contamination may occur. Short cooking times or other methods of preparing animal products (e.g., fermentation, drying, freezing, etc.) may sometimes not be sufficient to completely inactivate the parasites. The application of HHP represents one option for inactivation of parasites among other technologies (Gérard et al., 2019; Franssen et al., 2019), which at the same time preserves the desired degree of food freshness.

4.4.1 | Inactivation of parasites by HP

Research on inactivation of parasites by HHP has been primarily conducted on *Anisakis simplex* (roundworm), *Trichinella*, *Toxoplasma*, and *Cryptosporidium parvum* (protozoa) (online Appendix, Table 6). In one study, *Anisakis* larvae were isolated from fish tissue and examined for their mobility, as an indicator of survival, after HHP treatment of up to 200 MPa for 10 min, at 0 and at 15°C. The larvae were successfully inactivated at pressures above 140 MPa. However, at pressures below 140 MPa, the treatment time had to be extended by up to 1 hr to ensure the successful inactivation. Repetitive pressure cycles increased the success of the process when compared to a single pressure treatment and similar treatment time (Molina-García & Sanz, 2002). Eggs from *Ascaris suum* (porcine roundworm) were treated at different pressure levels, and at pressure higher than 300 MPa, multiplication of cells and thus the development of eggs was prevented. Below that pressure level, depending on the magnitude of the pressure, the percentage of developed eggs ranged from 2% after treatment at 250 MPa, to 98% after treatment at 138 MPa (Rosypal et al., 2011).

The effects of HHP (100–550 MPa, 1 min) on viability of *Toxoplasma gondii* oocysts were studied in feeding trials on mice. Oocysts treated at pressures of over 340 MPa were not infectious for mice, whereas untreated oocysts, or those treated at pressures below 270 MPa, caused acute toxoplasmosis. Examination under a light microscope revealed no structural changes in the oocysts after treatment at pressures of up to 550 MPa (Lindsay et al., 2005). An extended study investigated the effects of HHP treatment (100–400 MPa for up to 90 s) on viable tissue cysts of *Toxoplasma gondii* VEG (type III) in minced pork. The tissue cysts treated at pressures of over 300 MPa did not lead to infection in mice. Treatment at pressures of below 200 MPa resulted in infection regardless of the treatment time (Lindsay et al., 2006). Raspberries were inoculated with 5×10^4 oocysts of *Toxoplasma gondii* VEG (type III) and treated at pressures of 100–500 MPa for 60 s. The pressure of 340 MPa, applied for 60 s, was needed to prevent infection with the inoculated samples (Lindsay et al., 2008).

In another study, *Cryptosporidium parvum* oocysts were suspended in apple and orange juice, and subsequently treated at 550 MPa for up to 120 s. The results indicated inactivation of *C. parvum* oocysts by HHP by more than 3.4 log after 30 s. Samples treated with HHP for 60 s and longer exhibited no infectivity (Slifko et al., 2000).

To the best of the authors' knowledge, there are no tests reported in the literature for infectivity of *Cyclospora cayetanensis* (protozoa), which is detectable in foods that have been fecally contaminated and have caused intestinal infections. As the *C. cayetanensis* is difficult to study, as humans are its only known host, *Eimeria acervulina* (poultry coccidiosis) is suggested as a surrogate for this parasite, due to their similarities in morphology, life cycle, and genetics (Lee & Lee, 2001; Reiman et al., 1996). In one study, raspberries and basil were inoculated with different concentrations of sporulated *E. acervulina* oocysts (10^4 and 10^6 oocysts) and treated at 550 MPa at 40°C for 2 min. Oocysts isolated from the treated products were fed to broilers. The broilers were asymptomatic and did not excrete oocysts (Kniel et al., 2007).

The effects of HHP between 140 and 550 MPa for 1 min on the infectivity of *Encephalitozoon cuniculi* spores (encephalitozoonosis in rabbits) was studied *in vitro* on host cells. No effect on the infectivity of spores treated at 140 MPa was observed. The spores treated at pressure higher than 200 MPa exhibited a reduction in infectivity. After treatment at pressures higher than 345 MPa, the spores were no longer able to infect the host cells. No morphological changes were observed in pressure-treated spores inspected by transmission electron microscopy (Jordan et al., 2005).

4.4.2 | Methods for detecting inactivation

The infectious unit for parasites can be an individual (e.g., amoebae), an egg, or a larval stage (helminths), or even four to eight individuals (mature oocysts). For parasites that form tissue cysts, an infectious unit (the tissue cyst) can, therefore, consist also of a few to approximately 1000 individuals per tissue cyst (e.g., *Toxoplasma*). Due to the varying infectious units, logarithmic inactivation processes can only be described in case the underlying infectious unit is named (e.g., tissue cyst, cyst, oocyst, and egg). Unlike bacteria, parasites in or on food do not grow or multiply during storage. Therefore, even a reduction of as low as two or three orders of magnitude can be significant for parasitic contamination (Franssen et al., 2019).

The current standard method for assessing the inactivation of parasites relies on determination of infectivity by means of a bioassay. However, using laboratory animals for this purpose is considered controversial. In few recent studies, infectivity has been investigated using substitute indicators. Such validated indicators could be, for example, a parasite's loss of developmental ability, a microscopic assessment of motility or morphological integrity, or molecular biological methods for assessing genetic activities (Rousseau et al., 2018).

5 | EFFECTS OF HP ON CHEMICAL AND STRUCTURAL PROPERTIES OF FOODS AND FOOD CONSTITUENTS

Effects of HHP on the quality of foods and food constituents may result from the direct impact of HP on ingredients, or may be caused by its influence on the course of chemical reactions, which result in changes of the chemical composition. When studying cell-structured food systems, it is also important to consider the potential impact of HHP on cells and tissues. The application of HP may have indirect effects on the stability of ingredients; on the other hand, cell or tissue deterioration may also increase the extractability of constituents and thus impact the results of analytical determinations. The effects of HHP on key chemical reactions, selected food constituents, and allergenicity are discussed below.

5.1 | Effects of HP on chemical reactions

According to Le Chatelier's principle, reactions with a negative activation volume, for example, a number of polymerizations, cycloadditions, the formation of sulfonium or phosphonium salts and solvolytic reactions, are accelerated (Cheftel, 1995; Tauscher, 1995). Many of these well-known examples from organic chemistry are not expected to play an important role in foods. Over the course of years, significant amount of research has been performed and many model and food systems have been investigated. As a result, certain progress in understanding and validation of food-chemical reactions under pressure was achieved.

5.1.1 | Reactions of short-chain carbohydrates

Generally, carbohydrates remain fairly stable under HP conditions. The acid-catalyzed inversion of sucrose has been reported to be impeded under pressure (Röntgen, 1892; Sander, 1943), while polysaccharides can be degraded to a certain degree at very high pressures of over 1000 MPa (Kudla & Tomasik, 1992a, 1992b). On the other hand, an increased reactivity of carbohydrates can be expected, as the mutarotation velocity is higher under pressure (Andersen &

Gronlund, 1979). As shown for glucose, both anomers, either starting with pure α or β , react faster toward the anomeric equilibrium at higher pressure. This reaction can only proceed via the open chain form, which is the most reactive state of a carbohydrate and therefore, a higher concentration of this carbohydrate configuration under pressure can be assumed. Nevertheless, the influence of pressure on the rearrangement and degradation of saccharides is an open topic and currently no systematic investigations on this subject are available. Lactose was shown to be prevented from isomerization to lactulose by pressure in alkaline solution (Moreno et al., 2003). However, in milk, as a more complex system, Martínez-Monteagudo and Saldaña (2015) reported an activation volume of -7.5 cmş/mol for the isomerization of lactose to lactulose, and presented a mathematical model for this process. Once formed in the degradation of short-chained carbohydrates, dicarbonyls are transformed under pressure to yet unknown products (Schwarzenbolz et al., 2017; Schwarzenbolz & Henle, 2010), no matter whether proteins or amino acids are present or not. One possible route of the reaction is an enhanced aldol condensation, which is speculated from the formation of volatiles to be accelerated by pressure (Hill et al., 1999; Schieberle et al., 2005). Furthermore, a decomposition of certain volatiles like furanones and 2-acetyl-1-pyrrolin at HP could be confirmed (Schieberle et al., 2005). In proline-glucose mixtures, this led to a change of the flavor quality from popcorn-like to caramel-like. The authors explained their observation with the HP-induced formation of glucose degradation products like 2-oxopropanal, which subsequently undergo pressure-enhanced aldol condensation to form caramel-like smelling odorants.

The behavior of sugar, as mentioned above, may be of practical relevance. For example, the application of pressure prior to the ripening period of rice wine led to a faster decomposition of free saccharides and amino acids, indicating a higher reactivity of sugars, and in consequence to a shorter aging time to receive an oenological equivalent product (Tian et al., 2016).

Currently, no further information on the process-induced influence of pressure on caramelization (i.e., degradation reactions of sugars in the absence of protein or amino acids) is available. Nevertheless, the behavior of short-chained carbohydrates and their breakdown products are relevant for the outcome of Maillard reactions (MRs).

5.1.2 | Maillard reactions under HP

The term "Maillard reaction" (MR) refers to complex reaction cascades between reducing sugars and amino acids or their corresponding polymers, which has been subject of research for more than 100 years (Hellwig & Henle, 2014). Due to the complexity, the whole system of reactions was divided into three segments named early, advanced, and final stage (Hodge, 1953). At present, the information on the MR at HP in complex food systems is scarce; however, some reports can highlight the overall tendencies.

During the course of thermal food treatment, the MR is responsible for flavor formation, color development (nonenzymatic browning), and degradation of essential food ingredients, like amino acids through, for example, glycation of lysine residues. Furthermore, process-induced contaminants like furan (Yaylayan, 2006), acrylamide (Tareke et al., 2002), and 5-hydroxymethylfurfural (HMF) may be formed during food processing, and the physiological consequences of so-called advanced glycation end products (AGEs), even termed "glycotoxins" (Koschinsky et al., 1997), are still a matter of debate (Henle, 2007).

Thus, the influence of pressure on the MR is discussed on the following points:

- Short-chained carbohydrates and their degradation products (see above)
- Lysine residues in their reaction with carbohydrates and dicarbonyls
- Arginine residues in their reaction with carbohydrates and dicarbonyls

First experiments on classical MR systems have been reported by Tamaoka et al. (1991). In these experiments, no influence of pressure up to 400 MPa on the initial condensation reaction in the course of the MR could be observed. Subsequently, the authors measured the formation of "browning," that is, the increase of UV absorption at 420 nm, of mixtures of glyceraldehyde, glycolaldehyde, or xylose with amino acids, and reported a slower development under pressure. An activation volume of 13–27 ml/mol was calculated. From their results, the authors drew the conclusion that the early stage is not or only little affected, while the melanoidin formation at the final stage is impeded by pressure.

More in detail, Isaacs and Coulson (1996) provided activation volumes of -14 cmş/mol for the initial imine formation (condensation), 8 cmş/mol for the Amadori rearrangement, and 17 cmş/mol for the decomposition of the Amadori compound in model systems. They could also confirm the retardation of the final melanoidin formation, thus the accumulation of Amadori products during HPT is speculated. Subsequently, the extent of browning reactions was shown to depend on the initial pH value (Hill et al., 1996). While it is slowed down at acidic pH, a more alkaline environment is promoting browning reactions. The latter results were reproduced in systems containing glucose and

lysine (Moreno et al., 2003). Based on the analysis of furoylmethyl lysine, that is, furosine, as well as on UV spectrometry, at a starting pH < 8, the initial phase of the MR was not affected or decreased (depending on the buffer), while the concentrations of browning products are lowered by pressure. At a pH of 10.2, the relations are reversed, now leading to increasing amounts of early and advanced Maillard products.

The value of statements, which validate the overall course of the MR without characterizing individual products, was questioned with lab-scale investigations of model mixtures. This was done especially for mixtures of lysine and/or arginine residues with saccharides or dicarbonyls. Starting points were experiments on pentosidine, an AGE formed in a condensation reaction between arginine, lysine, and a pentose. Its concentration was reported to rise by a factor of 10 when comparing setups at atmospheric pressure to setups at 600 MPa (Schwarzenbolz et al., 2000). In contrast, pyrraline, an AGE which originates from a condensation between a lysine residue and 3-desoxyglucosone, is suppressed almost completely at 600 MPa, while at atmospheric pressure, it is an indicator for the advanced MR (Schwarzenbolz et al., 2002). Regarding individual reaction products, fructoselysine, the Amadori product in a reaction between lysine and glucose, is of particular importance, as it represents by far the most abundant Maillard product in food (Henle, 2003). Despite advances in analytical equipment, there are still no clear results about the fructoselysine formation under HP. Ma et al. (2017) discussed an enhancement under pressure, while in contrast, Moreno et al. (2003) postulated a decreased advanced MR at a starting pH of 9.8. Besides this, the authors showed that arginine is also a potent partner for reactions with saccharides and concluded that there is no general rule for the HP effects on MR systems. This led to the opinion that the influence of pressure on Maillard-type reactions is not equivalent to temperature and hence results cannot be transferred directly, for example, to maintain Hodge's three-stage scheme.

Studies on reaction systems containing lysine and a carbonyl component (glyoxal) exhibited positive activation volumes when reacted under differentpressures. For the formation of typical products like N^{ε}-carboxymethyllysine (CML), an activation volume of 5.4 cmş/mol, and for glyoxal-lysin-dimer (GOLD), a value of 9.9 cmş/mol was determined, respectively (Schwarzenbolz et al., 2017). This effect was explained by a shift of the equilibrium of lysine's amino group toward its protonated form and an enhanced side reaction (e.g., degradation or aldol condensation) of the dicarbonyl compound by pressure (Schwarzenbolz & Henle, 2010). Additional evidence for a reduced reactivity of lysine residues under pressure was provided by Buckow et al. (2011). Incubating bovine serum albumin with glucose at elevated temperatures (60–132°C) led to higher amounts of residual free amino groups with increasing pressure. These observations gained additional support by the measurement of volatile compounds from MR systems. In mixtures containing either xylose and lysine (Bristow & Isaacs, 1999) or glucose and lysine (Hill et al., 1999), respectively, HPT generally led to a decreased formation of volatiles. Independently of the individual end products (furanones in xylose/lysine and pyrazines in glucose/lysine systems), the concentrations were reduced drastically by pressure. Additionally, an acceleration of the furanone's decomposition was observed.

Kebede et al. (2013) reported in an untargeted approach with statistical evaluation for the analysis of volatiles from different HP and high temperature-treated vegetables, that pressure is suppressing reactions, which lead to the formation of typical MR volatiles, like "Strecker aldehydes" (Kebede et al., 2017) and furanic compounds, while increasing amounts of odorants, that is, aliphatic aldehydes and ketones, which may be attributed to the degradation of unsaturated fatty acids, can be found.

Regarding arginine residues, Alt and Schieberle (2005) reported that the reaction with glucose is influenced by pressure. Their experiments revealed pressure-induced formation of carboxyethylarginine (CEL) and increased concentrations of hydroimidazolones, namely the methylglyoxal derivative MG-H1. In contrast to lysine, arginine is well able to react in higher amounts with higher pressure. Due to the pKa of 12.6, arginine is protonated, nevertheless, the guanidine group provides free pairs of electrons, which are able to undergo Maillard-like reactions.

5.1.3 | Oxidation of lipids under HP

Lipid oxidation follows a radical chain reaction whose stages can be influenced by pressure (Tauscher, 1995). In experiments with linoleic acid, the estimation that the initial radical formation is retarded, while the propagation step is accelerated, could be confirmed (Martinez-Monteagudo & Saldaña, 2014). Reactions leading to a termination of the radical chain are controlled by diffusion, and hence are expected to be hindered by pressure. In consequence, the initial quality prior to the technological treatment and the presence of pro-oxidants are crucial for the effects of pressure. Often, in HP-treated foods, the shelf-life is reduced due to lipid oxidation, as a result of the liberation of pro-oxidants like metal cations from the food matrix (Bolumar et al., 2012; Buckow et al., 2013; Cheah & Ledward, 1996; Segovia Bravo et al., 2012; Simonin et al., 2012). It is noteworthy that, at least in meat and meat products, there is a close relation between protein and lipid oxidation, which may be challenging in terms of product

quality (Guyon et al., 2016). As many parameters like pH, water content, antioxidants, spectrum of fatty acids, or enzymatic activity are able to interfere with the outcome of lipid oxidation, the comparison of investigations on food systems is often limited.

5.1.4 | Food process contaminants

Formation of food process contaminants in traditional food processing is mainly dependent on the intensity of a heat treatment. Reduced heat load during the HHP treatment consequentially leads to lower concentration of process contaminants compared to a traditional heat treatment. As an alternative to thermal sterilization, HPTS is of major interest. There are some indications on different and slowed down MR pathways during HP treatment, depending on the pH value, treatment time, and pressure level, which can lead to lower formation of AGEs.

Acrylamide formation was examined in incubation mixtures consisting of the typical precursors asparagine and glucose (De Vleeschouwer et al., 2010). In different systems (high and low moisture), it could be clearly shown that pressure is suppressing the formation of acrylamide. As acrylamide and furan may also be promoted by lipid oxidation products (Keramat et al., 2011), it is noteworthy that these processes may also be considered with respect to food safety. Above this, main intermediates in lipid oxidation are carbonyls, which can act similar to carbohydrates in Maillard-like reactions. Pressure has also impact on the formation of furan. As summarized by Sevenich et al. (2013), there are steps in the reaction pathway toward furan, which are hindered by pressure. Although, in consequence, the furan concentration in HPT food is expected to be lower than after conventional heating, the question about furan's precursors remains open.

In solutions of whey protein with glucose or trehalose at different pH, both browning and several indicators (furosine, CML, and CEL) are reduced comparing HPT to high-temperature treatment (Ruiz et al., 2016). For the HMF, for which toxicological relevance is still questionable (Abraham et al., 2011), one study reported that pressure could inhibit degradation of the Amadori rearrangement product, which leads to a significant reduction of MR intermediates, browning intensity, and HMF content (Guan et al., 2011). The comparison of pressure and temperature treatment of mango nectar showed no significant differences. After both treatments, decreases in glucose and fructose were observed, while the concentration of sucrose remained constant and the concentration of HMF increased (Liu et al., 2014). Also, no significant differences in the concentrations of the sugars were observed between pressure and temperature-treated mango nectar. Solely the HMF content was slightly lower after pressure treatment and subsequent storage at 4°C. But during storage of the nectar, especially at 25°C, it became obvious that pressure has left residual invertase activity, which led to a degradation of sucrose in favor of glucose and fructose. This later effect was already described before on strawberry jam (Kimura et al., 1994).

For the formation of polycyclic aromatic hydrocarbons (PAHs), drastic temperature conditions are required, for example, incomplete combustion that cannot be achieved in commercial use of HHP or HPT. Thus, formation of these compounds as a result of HHP treatment is not to be expected (Segovia Bravo et al., 2012).

For N-nitroso compounds, there is a single report that these may be formed to a higher extent in the presence of nitrite and secondary amines under increased pressure conditions (Segovia Bravo et al., 2012). Unfortunately, a validating confirmation of this observation is missing.

5.2 | Effects of HP on food constituents and food structure

In recent years, many studies have dealt with the influence of HHP on food ingredients and structures, some of which also concern aspects of food safety (Barba et al., 2015; Georget et al., 2015; Oey et al., 2008). In general, it has been reported that quality aspects of food treated by HHP are not significantly affected compared to untreated sample, or less affected compared to conventional thermal processing. It is generally recognized that HHP is not expected to affect the primary structure of food constituents within usual commercially used pressure ranges. These ranges are far below 2 GPa and not sufficient to modify covalent bonds, due to their very low compressibility (Aertsen et al., 2009). In contrast, the spatial structure of macromolecules is influenced by HHP due to its compressibility and the resulting dynamic behavior of macromolecules under pressure (Balasubramaniam, 2016). Therefore, HHP can be used not only for preservation of food, but also for the targeted modification of food structures. Online Appendix table 7 summarizes examples of studies investigating the impact of HHP on food constituents. A condensed summary with a focus on food safety aspects is given in the following section.

5.2.1 | Impact of HP on secondary plant metabolites

Secondary plant metabolites are low-molecular food constituents that are not directly affected by HP treatment. For

example, chlorophyll remains very stable under HHP conditions at room temperature (Butz et al., 2002), and a significant degradation starts only in combination with elevated temperature (> 50°C) (Van Loey et al., 1998). However, the effects of HP treatment on the cell structure of plant tissue, and thus on enzymatic and nonenzymatic processes, may result in effects on secondary plant metabolites.

Depending on the pressure level, different phenomena may occur. For pressure levels below 100 MPa, stress reactions may be induced in vital plant tissue leading to changes in secondary plant metabolite concentration. Pressure levels between 150 and 200 MPa may result in disintegration of the cell membrane and changes of the cell wall (Rux et al., 2020). Increasing structural changes occur at increasing pressure levels above 200 MPa. Depending on the matrix, secondary metabolites that are attached to the polymeric structure of the cell wall or present in cellular organelles will be released to a larger extent but at the same time may be more susceptible to enzymatic or oxidative degradation (Serment-Moreno et al., 2017).

In some cases, an increase in the concentrations of phenols and other bioactive compounds was observed after HHP treatment (Sánchez-Moreno et al., 2005; Szczepańska et al., 2020) which, according to the different authors, is primarily a result of improved extractability due to cell disruption after pressure treatment (Gómez-Maqueo et al., 2020). Nutritionally valuable substances that may be released in larger quantities were shown to be subject to enzymatic or nonenzymatic reactions, particularly oxidation processes, during subsequent processing steps (Wang et al., 2018). However, their loss is not relevant to the safety of the products. The cell disruption could also lead to a higher extraction of antinutritive substances, such as trypsin-inhibiting malanoidins from black garlic (Zhao et al., 2019), which on the other hand may have an impact on product safety.

Controversial results on the nutritional effects of secondary plant metabolites led to discussions on the risks and benefits of these substances. In many cases, there is no clear information on concentration thresholds with the positive or negative effects of various secondary metabolites on health, even for established conventionally treated food products. Therefore, on the basis of the present knowledge, no general conclusions regarding product quality and safety can be drawn regarding the effects of HHP on this group of constituents.

5.2.2 | Impact of HP on vitamins

Generally, vitamins are considered to be pressure-stable, due to the lack of spatial structures and the pressure stability of covalent bonds (Mahadevan & Karwe, 2016). However, significant degradation rates under pressure were observed for folates, which were explained at the molecular level related to negative activation volumes of folate vitamers (Verlinde et al., 2009). Higher concentrations of carotenoids, chlorophylls, and tocopherols were found in plant-based foods after HP treatment, compared to untreated material. This indicates an improvement in extractability of these compounds after HHP (Arnold et al., 2014; Westphal et al., 2018).

Existing studies on HHP treatment often draw comparisons with either untreated starting material or thermally treated product. The latter can be used as comparator only to a very limited extent, as the comparability of the two treatments is challenging due to different results in microbial inactivation and product stability in general. A reduction in the vitamin content could be due to increased cell disruption, allowing for enhanced oxidation or enzymatic activity. On the other hand, some studies attributed the observed increase in vitamin content after HHP treatment to cell disruption, leading to an improved extractability of the constituent and thus a higher analytically determined concentration. From the safety perspective, a comparison to untreated and conventionally treated products is relevant for critical process-induced degradation products, and high vitamin losses of HHP-treated products would also be considered as a negative effect. Future studies reporting on these two aspects would need to be reviewed carefully with regard to food safety concerns arising from HHP treatment.

5.2.3 | Impact of HP on polysaccharides

Pressure-induced modification of physical and chemical properties of polysaccharides is known. This may include a change in water-binding properties or gel formation that can occur during HP treatment, or reduction of gelatinization temperature with relevance for the thermal behavior of ingredients and related changes of the water activity of systems (Bolumar et al., 2016). HHP has a large influence on the structure of the starch granules (Słomińska et al., 2015). Although there are differences in the pressure sensitivity of starch granules, depending on the source and/or the structure (Le Bail et al., 2013), gelatinization of starch by HHP is also achieved at low temperature, as amorphous and crystalline regions of the granules are modified under pressure (Pei-Ling et al., 2010; Yamamoto & Buckow, 2016). Changes at the molecular level at pressures outside of technologically relevant range (>650 MPa) have also been reported in the literature. Depending on the type and origin of starch, its structure may vary and thus, both 1,4 and 1,6 glycosidic bonds can be affected by the pressure, where hydrolysis of these bonds can lead to size reduction of the starch molecules (Szwengiel et al., 2018). This may also

have effects on the release of starch-associated molecules as described, for example, in thermal processing (De Girolamo et al., 2016). However, this has not yet been studied for HHP treatments.

5.2.4 | Impact of HP on proteins

Pressure-induced denaturation of proteins is well studied and is recognized to be reversible or irreversible. It can also be accompanied by aggregation or gel formation. Changes in protein conformation under HP result in a smaller specific volume, and at pressures below 200 MPa may involve the dissociation of oligomeric proteins (Yang & Powers, 2016).

Literature data on chemical stability of proteins under HHP are scarce. It is known that muscle proteins can undergo oxidative processes (Lund et al., 2011). Few other literature sources that specifically studied protein oxidation under HP reported either increase in oxidation (Fuentes et al., 2010) or no effect (Cava et al., 2009) after pressure treatment. A study on HHP treatment of pork batters conducted by Villamonte et al. (2017) found an increase in protein carbonylation and a decrease in sulfhydryl groups, but no further enhancement of protein oxidation during subsequent storage was reported. The protein oxidation in the treated pork batters was associated with an increase of the content of nonheme iron after HHP. At present, no fundamental conclusions can be drawn from the contradictory information available. However, there are currently no indications that changes in the protein's status negatively affect the nutritional or microbial product safety.

5.2.5 | Contaminants and residues

Monochloropropanediols (2-MCPD, 3-MCPD), their fatty acid esters, and glycidol fatty acid esters can enter foodstuffs as process contaminants during refining of edible fats and oils. Studies on behavior of 3-MCPD demonstrated no significant changes in concentration under HP conditions; therefore, neither formation of new nor degradation of these compounds is to be expected in the course of HPT treatment (Sevenich et al., 2013; Sevenich et al., 2015).

Certain studies reported a decrease of mycotoxins in food products after HHP treatment, but so far, there is no clear information on the underlying mechanisms of reduction. In general, the relevance of such observations may be questioned, as it is not the processing, but monitoring of raw materials that is decisive for the avoidance and reduction of appearance of mycotoxin in foods (Avsaroglu et al., 2015; Hao et al., 2016; Tokuşoğlu et al., 2010). A change in binding and release of mycotoxins, as described for thermal processes under certain conditions (De Girolamo et al., 2016; Rychlik et al., 2014), has not yet been studied for HHP.

5.2.6 | Impact of HP on cell and tissue structure

In most cases, HHP is accompanied by a change in the texture of cell-structured food systems. The main cause is the deteriorating effect of HP on cell membranes and cell walls; this phenomenon is intensified in products with a high proportion of air-filled pores, owing to the differences in compressibility of the tissue materials and the entrapped air. The consequence for plant-based products is a softening of the tissue and destruction of intracellular structures (Gonzalez & Barrett, 2010). As a result, release of cell contents and increased enzyme-catalyzed and oxidation reactions might occur. Similar cell disruption effects are observed with other mechanical or thermal processes. In thermal processes, however, cell disruption is a consequence of the temperature increase, which can simultaneously lead to inactivation of enzymes and thus to a reduction of enzymatic reactions. In carrots, the HHP- and the HPT-process were compared to the equivalent thermal process with comparable microbiological inactivation rates. The HHP-process resulted in less damage of tissue structures than the comparable thermal process. However, the damage of tissue structures caused by the HPT-process was comparable to that observed after application of the equivalent comparable process (Knockaert et al., 2011; Vervoort et al., 2012).

However, cell disruption in general can contribute to destabilization and thus possibly to acceleration of degradation processes. On the other hand, improved extractability of food constituents after high-pressure treatment is described and often reported in the literature as higher concentration of these compounds after the HHP. There is also a possibility that an increase in secondary metabolites may be attributed to a stress reaction of the vital cell.

The changes described above may occur to varying degrees, depending on the compound under consideration and its integration into the cell structure, tissue properties, intensity of the high-pressure treatment, degree of the resulting cell disruption, and relevant enzyme reactions and degradation processes. Resulting effects on food safety aspects have not been described yet.

5.3 | Impact of HP on enzymes

In addition to the inactivation of microorganisms, the application of HP in food processing also aims to

inactivate undesired enzymes present in foods. Potential concerns are related to the impact of HP on enzyme activities (inactivation or enhancement) and on substrate specificities.

5.3.1 | Impact of HP on enzyme activities

There are enzymes that are significantly inactivated as a result of time/pressure combinations commonly applied in food processing. On the other hand, there are several enzymes for which enhancements of their activities upon HP treatment have been observed (Eisenmenger & Reyes-De-Corcuera, 2009).

Both inactivation and enhancement are influenced by properties of the food matrix. However, data allowing generic conclusions or predictions regarding the impact of a specific food on the behavior of an enzyme under HP are not available. The food enzymes studied so far are mainly related to quality aspects of foods. Thus, changes induced by HP, for example, increased activity of polyphenoloxidase, may have undesirable consequences for the quality of the food. However, food safety-related issues resulting from the change of enzyme activities upon HP treatment have not been reported.

5.3.2 | Impact of HP on substrate specificities

The application of HP may induce conformational changes of substrates resulting in changed accessibility of functional groups required for the enzymatic catalysis. For example, native bovine ß-lactoglobulin (bLGL) is no substrate for microbial transglutaminase (mTG). However, after incubation of the protein with mTG for 1 hr at 40°C at 400 MPa, four out of nine glutamine residues were identified as accessible for the mTG-catalyzed reaction. This indicated partial unfolding of bLGL under pressure and exposure of previously inaccessible glutamine residues (Partschefeld et al., 2007). Similarly, hen egg white lysozyme (HEWL) does not represent a substrate for mTG at atmospheric pressure. However, after incubation of HEWL with mTG under HP (400–600 MPa) for 30 min at 40°C, the formation of an isopeptide crosslink between lysine in position 1 and glutamine at position 121 was observed, indicating a pressure-induced unfolding of the protein (Schuh et al., 2010).

So far, no changes of substrate specificities owing to HP-induced modifications of enzymes have been reported.

5.4 | Impact of HP on allergenicity

5.4.1 | Effect of HP on protein structures with possible effects on allergenic potential

As already mentioned, HHP treatment affects noncovalent bonds (e.g., ionic, hydrophobic, and hydrogen bonds), thus inducing changes in the secondary, tertiary, and quaternary protein structure. This can lead to reversible and in part, irreversible unfolding, denaturation, aggregation, and gelatinization of proteins (Huang et al., 2014; Somkuti & Smeller, 2013; Vanga et al., 2015). As a consequence, in particular structurally defined allergenic determinants (epitopes) of proteins can change, which can influence their ability to bind allergen-specific antibodies of the IgE isotype. Consequently, the allergenic potential may change after HHP treatment.

5.4.2 | Criteria for the selection of studies

Isolated structural analyses of technologically (e.g., thermally or by HHP) treated allergenic proteins usually do not allow for direct assessments on the type and extent of the resulting allergenic potential. Consequently, studies that exclusively investigated structural changes after HHP treatment were not considered in this review. Similarly, studies on the antigenicity of allergenic proteins or protein fractions using allergen-specific antibodies generated in animals, but without characterization regarding the recognition of human IgE-binding determinants, were not considered in this review. Consequently, only studies that investigated the influence of HHP treatment on allergenic food or food proteins using suitable allergenic parameters were evaluated. These include (1) qualitative and quantitative binding properties of allergen-specific IgE, (2) biologically functional activation of effector cells, such as basophils and mast cells, and (3) allergic skin provocations, as well as (4) allergic reactions after oral intake or provocation. The latter *in vivo* tests have the greatest significance with regard to the triggering of allergic reactions, whereas pure IgE-binding data can only provide indications of a possibly altered allergenic potential in the case of pre-existing sensitization.

5.4.3 | Review of the selected studies

Most of the considered studies are limited to description of the pure IgE-binding properties of plant and animal-based food and allergenic proteins after HHP treatment,

often in the pressure range of 100 and 800 MPa, and application times of 5–60 min. Plant seeds (soy bean, almond, ginkgo, wheat, and buckwheat), fruits and vegetables (apple, pineapple, carrot, and celery), cow's milk and hen's eggs, as well as meat (beef), fish (perch and large-mouth bass), shellfish, and mollusks (shrimp and squid) were often studied (online Appendix, Table 8: References 1–34). In addition to studies using only HHP as a technological process (online Appendix, Table 8: References 1, 3, 6–12, 14–17, 21, 24–26, 28–32, and 34), data on combined HHP and heat treatment (50–115°C) (online Appendix, Table 8: References 12, 13, 26, and 33), or HHP and enzymatic hydrolysis (pepsin, trypsin, chymotrypsin, papain, alcalase, neutrase, and corolase) (online Appendix, Table 8: References 2, 4, 5, 18–20, 22, and 23) were considered. The vast majority of studies were performed *in vitro* as IgE-binding studies with serum IgE of allergic subjects (online Appendix, Table 8: References 1, 2, 5–9, 12, 13, 15–22, and 26–34) or by means of human basophil activation or mediator release experiments (online Appendix, Table 8: References 11, 14, 15, 25, 28, 29, and 30). Few *in vivo* studies (skin and oral provocation) have been conducted in allergic subjects (online Appendix, Table 8: References 10, 11, and 14). Likewise, only few studies have been conducted in murine models (online Appendix, Table 8: References 3, 4, 23, 24, and 33). Aqueous or buffered food systems (including juices) and total protein extracts (online Appendix, Table 8: References 1–8, 11, 12, 14, 17–19, 31, and 32), often single allergens (wheat alpha-amylase inhibitor, apple Mal d 1, carrot Dau c 1, celery Api g 1, milk β-lactoglobulin, hen's egg ovomucoid and ovalbumin, bovine serum albumin, bovine gamma globulin, shellfish and mollusk tropomyosin) (online Appendix, Table 8: References 9, 11, 14, 16, 20–23, 25, 27–30, 33, and 34) and less frequently whole foods (apple and apple skin, celery tuber, milk, hen's egg powder in minced beef) (online Appendix, Table 8: References 10, 13, 15, 24, and 26) were studied.

5.4.4 | Allergenicity assessment

In most cases, HHP as a sole technological process led to no (online Appendix, Table 8: References 1, 2, 6, 11, 12, 14, 16, 17, 28, 31, and 32) or only a slight reduction (online Appendix, Table 8: References 7, 9, 13, 15, 26, 29, 30, and 34) in IgE-binding properties. In a single study on milk proteins, HHP treatment resulted in a slight increase in IgE-binding, with statistical significance only at a pressure of 200 MPa (online Appendix, Table 8: Reference 21). HHP combined with thermal treatment predominantly led to a reduction in the IgE-binding capacity (online Appendix, Table 8: References 12, 13, 26, and 33). The combination of HHP and protease(s) resulted predominantly in a reduction in IgE-binding (online Appendix, Table 8: References 2, 4, 5, 18, 19, 20, 22, and 23), whereby HHP treatment was primarily used for accelerated proteolysis. Because HHP as a sole treatment reduces IgE-binding only slightly (if at all), the reduced IgE-binding in combination with proteases or heating is due to (accelerated) proteolysis or heating of the allergens. In experiments on *in vitro* (human) basophil activation or mediator release, HHP as a sole treatment process led to no reduction or only a slight reduction in allergenic potential (online Appendix, Table 8: References 11, 14, 25, 28, 29, and 30).

HHP-treated apples showed a reduced allergenic potential in *in vivo* skin tests of birch-pollen-allergic subjects with apple allergy (Meyer-Pittroff et al., 2007), whereas the allergenic potential of HHP-treated apple juice was not reduced (Houska et al., 2009). In both cases, the authors referred to Mal d 1, the thermolabile major apple allergen, which is associated with birch-pollen allergy. However, individual data on allergen-resolved sensitization patterns are lacking; therefore, conclusions with regard to allergen-related patterns of allergic response cannot be drawn from the contradictory results. In another study, HHP-treated carrot juice did not exhibit reduced allergenic potential in the skin test conducted on birch-pollen allergic subjects with carrot allergy (Heroldova et al., 2009). HHP-treated apples were well tolerated in an oral provocation of 19 birch-pollen-associated apple allergic subjects (Meyer-Pittroff et al., 2007). The authors suggested the inactivation of the birch-pollen-associated major apple allergen Mal d 1 as the cause of reduced allergenic potential. However, HHP treatment of Mal d led to an increase of *in vitro* basophil activation , which indicates an increase in allergenicity. In the oral provocation of 10 allergic subjects having birch-pollen-associated apple allergy, 10 subjects presented allergic reactions to untreated apple juice and five to the HHP-treated juice (Houska et al., 2009). In the oral provocation of five carrot allergic subjects, five presented allergic reactions to untreated carrot juice and three presented allergic reactions to the HHP-treated juice (Heroldova et al., 2009). There was a tendency for birch-pollen-associated allergies to apple or carrot to exhibit no or only a slight reduction in allergenic potential after the HHP treatment.

6 | IMPACT OF HP ON PACKAGING MATERIALS

In order to assess HP effects on packaging materials, chemical and physical changes of the properties of polymers under pressure should be studied.

In recent years, there has been a significant knowledge gain regarding packaging materials that are suitable for the HP treatment of food. Review articles summarizing

TABLE 2 General requirements for packaging materials

Integrity requirement[a] (Max. expected pressure/temperature)	HP-LT (600 MPa/80°C)	HP-HT (800 MPa/133°C)	Sterile retort pouch[b] (0.2 MPa/133°C)
Visual integrity	No delamination or blistering	No delamination or blistering	No delamination and no blistering
Oxygen permeability(Max. deviation 12%)	Product-dependent	0.5–1.0 ml/m^2/day (for commercial products) 0.06 ml/m^2/day(U.S. military products)	0.5–1.0 ml/m^2/day (for commercial products) 0.06 ml/m^2/day(U.S. military products)
Water permeability(Max. deviation 12%)	Product-dependent	0.01 g/m^2/day orproduct-dependent	0.01 g/m^2/day orproduct-dependent
Seal strength properties(Max. deviation 25%)	Material-dependent	Material-dependent	Seal strength 2–3.5 kg/100 mm; bond strength 150–500 g/10 ml; Burst test7.5 kg/15 mm seal
Physical strength (tensile, elongation, elasticity modulus)(Max. deviation 25%)	Material-dependent	Material-dependent	Material-dependent
Total migration of packaging components into food simulants	<10 mg/dm^2	<10 mg/dm^2	<10 mg/dm^2
Maximum headspace[ad]	Up to 30%	Up to 30%	Up to 30%
High thermal conductivity[d]	Not required	Required	Required

[a](Lambert et al., 2000a, 2000b); Headspace requirements as per Japanese standard.
[b](Venugopal, 2006).
[c]EUR-Lex - 31990L0128 - EN - EUR-Lex - europa.eu
https://eur-lex.europa.eu/legal-content/DE/ALL/?uri=uriserv:OJ.L_.1990.075.01.0019.01.DEU=uriserv:OJ.L_.1990.075.01.0019.01.DEU.
[d]Not yet defined as a standard selection criterion.
Adapted from Juliano et al. (2010).

the scientific status on the subject of HP treatment and packaging, for example, on general requirements for packaging materials for different HHP treatments, are already available (Juliano et al., 2010) (Table 2).

Packaging materials for food have to fulfill a number of integrity requirements that must be met before their use in various product applications. These include visual integrity, gas and water permeability, sealing and physical strength properties, and the migration of chemical substances into the packaged food. Evaluations of the visual integrity, gas permeability, tightness of sealing seams, and physical properties of packaging materials after HHP treatment are frequently discussed in the literature, but more detailed information is often missing to provide a complete picture of the suitability of various packaging materials.

General conditions for the selection of the optimal packaging materials suitable for HHP can be summarized as follows (Singh, 2017):

- Packaging films and containers are more compressible than water under HHP.
- Studies show that the oxygen barrier and the water vapor barrier in the HHP process generally even improve, given that the reduction of volume can lead to an increase in the crystallinity of the polymer molecules under HP.
- At 600 MPa, the volume of the water or food (where water is the major constituent) is compressed by around 15%; accordingly, the packaging must be flexible by at least this value, while taking the compression of any headspace gases present into account.
- Ethyl vinyl alcohol composites (EVOH composites) seem to be the best choice in terms of barrier properties. Laminates with aluminum, metallized films, and SiOx coatings seem to be less suitable. Glass or metal containers are not suitable.

6.1 | Changes observed in packaging materials under HP

Problems with multi-layer composite materials (multi-material and multi-layer structures), which can arise when used in HHP processes, have been reported many times.

In particular, phenomena like delamination to structural destruction, particularly of composites with aluminum or other inorganic components, such as Al, AlOx, or SiOx vapor deposition, are reported (Juliano et al., 2010; Mensitieri et al., 2013), and are described in the results of the CORNET AiF 26 N programme, "Packaging material for High-Pressure Treatment (HiPP).[1]"

In addition, headspace filled with gas must be avoided in the packaging; the differences in compressibility between water and gas can cause damage to the packaging. Also, the capacity of the high-pressure system can be better utilized, if headspace is avoided (Juliano et al., 2010).

6.2 | Mass transfer from packaging materials under HP

The availability of data in the literature on mass transfer from packaging materials to food (migration) is scarce for HHP. Some studies have examined the migration behavior of different packaging materials on a selective basis, but not comprehensively, and not always based on relevant legal designation on migration measurements and limit values (at least for the European Economic Area). In a classical HHP treatment, a distinction must be drawn as to whether a change in migration behavior is actually caused by the higher pressure applied or by the temperature increase that occurs due to compression (guideline value: 10°C temperature increase results in a doubling of migration [EC, 2011]).

There is evidence that migration from polypropylene (PP) and polyethylene terephthalate (PET) films in 10% ethanol is reduced under pressure compared to thermal treatment (Song & Koontz, 2016). The subsequent storage of the films treated by HHP did not reveal significant differences in migration compared to samples undergoing only thermal treatment. The authors considered food packaging materials commonly used to be safe for HHP application in terms of migration.

The migration behavior of plastics (composites) was also investigated within the scope of the CORNET project HiPP (2011). With one exception, the results of the overall and the specific migration tests were within the legal requirements. The migration of the leading substance (CAS 2082-79) of one plastic material (PETX12/PET23/PE50) was above the permitted limit. However, the assumption that increased crystallinity of polymers at HPs could possibly also lead to a decrease in the tendency to migrate was not confirmed. In principle, HHP conditions could promote densification of the amorphous domains of polymers, increase in melting and crystallization temperature, and change in the morphology of the crystalline and amorphous domains in polymer films (Mensitieri et al., 2013).

[1] https://www.fei-bonn.de/gefoerderte-projekte/projektdatenbank/cornet-aif-26-en.projekt

6.3 | "New" packaging materials and outlook

There are few studies on the HHP-suitability of "new" packaging materials made of renewable or biodegradable materials, such as polylactic acid (PLA) (Sansone et al., 2012). The effect of HHP on PLA has been analyzed in terms of structural/morphological changes (e.g., crystallinity, density, and orientation) and functional properties (e.g., melting behavior and gas transition temperature, permeability and solubility of gases and water vapor). It was found that HHP treatment does not significantly affect any structural or functional property of the treated material. In contrast, HPT sterilization promoted hydrolysis of the material, accompanied by an increase in crystallinity and a decrease in the density of the amorphous phase as a function of the temperature/pressure curve. These effects can lead to unacceptable levels of brittleness and turbidity in the material, making it unsuitable for HPT sterilization applications.

7 | LEGAL ASPECTS OF HP

Currently, four main European institutions are involved in development of laws at the EU level: (1) the European Parliament (EP), (2) the European Commission (EC), (3) the European Council, and (4) the European Court of Justice. Although not being an EU institution, the European Food Safety Authority (EFSA) is an agency that operates independently of the European legislative executive institutions and EU Member States. It was founded in 2002 to be a source of scientific advice and communication of the risks associated with the food chain. To carry out a risk assessment, EFSA often works closely with national competent authorities. Based on the EFSA's assessment, the EC and the EP make policy decisions on how to manage that risk. Accordingly, EFSA may identify that a certain food or a process represent a risk. It is on an EU or a member state level to consider this assessment, set policy or law, or manage that risk (Watkins, 2012).

According to the Regulation (EC) No 258/97 concerning novel foods and novel food ingredients (EC, 1997), a food or food ingredient should be considered as a novel food and subject to authorization, to which a production process has been applied not used in the EU prior to 15 May 1997, and where that process gives rise to significant changes in the

composition or structure of the foods or food ingredients, which affect their nutritional value, metabolism, or level of undesirable substances.

Whether a product produced by means of an HHP is subject to the Novel Food Regulation is a question of assessment on a case-by-case basis. For placing a food produced with "emerging" technologies (i.e., HHP) on the market, the applicant has to submit a request to a Member State in which the product is to be placed on the market, including studies carried out to demonstrate that the food complies with demanded criteria. Novel foods must be assessed in terms of health and authorized before they can be placed on the market. It is also necessary to ensure that consumers are not misled by the use of a novel food.

Novel foods that have received authorization are specified in the Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. The food included in the list may be placed on the market, if the conditions of use, specific labeling requirements, specifications, and other requirements indicated therein are complied with.

Food companies are responsible for their own products and are therefore responsible for verifying whether or not a food or food ingredient falls under the Novel Food Regulation.

However, companies which are uncertain as to whether a product is classified as a novel food may consult the competent authority of the Member State where they first intend to place the product on the market. Detailed contact details of the EU countries' authorities responsible for the consultation process on novel food status of a food are given on the EC website.[2] The specific details of this consultation procedure, including the information to be provided by the food industry operator, are laid down in the Implementing Regulation (EU) 2018/456 on the procedural steps on the consultation for determining novel food status.

The EC publishes information on the novel food status of products on its website.[3] In Germany, the enforcement of food law regulations is the responsibility of the competent authorities of the federal states.

In the United States, five organizations are dealing with food safety matters: the Food and Drug Administration (FDA), the Food Safety and Inspection Service (FSIS), the Center for Disease Control and Prevention (CDC), the U.S. Department of Public Health, and the U.S. Department of Agriculture (USDA). When introducing new processes, the food processors have to demonstrate that a certain technology delivers safe foods. Similar to thermal processing, validation studies have to be conducted for HHP to determine pressure-time conditions and to validate the HACCP plan that a treatment consistently achieves a minimum 5-log reduction of pertinent microorganisms for that type of a product, providing consumers an adequate level of protection from hazards related to the product, during its storage time under defined conditions. Process verification responsibilities for HHP, as a killing step for pathogens, are established in the FSIS Directive 6120.2. FDA does not consider HHP to be a validated process that can eliminate the spores of *Clostridium botulinum* in low acid products.

For preservation of low acid-foods, FDA issued no objections to two petitions for preservation of mashed potato and seafood using HPT process. Regarding ready-to-eat meat products, commercialization policies are established by the FSIS of the USDA, which in 2003 issued a letter of no objection for the use of HHP as an effective post-packaged intervention method to control *L. monocytogenes*, which is considered the pertinent pathogen in this food category (Stewart et al. 2016).

In Canada, novel food aspects are governed by Health Canada, working closely with the Canadian Food Inspection Agency (CFIA). In December 2016, there was a position published indicating that, based on sufficient knowledge and data available that HPP can be safely applied to food, HPP is no longer a novel process.[4]

Australia and New Zealand follow a similar approach as the United States, where food processors have to demonstrate that the HHP process will produce safe and suitable product, by conducting validation studies to confirm that parameters, such as pressure and holding time, are effective at reducing the microorganisms of concern to an acceptable level when applied to your product.[5,6] Responsible authorities issued guidance documents to support and help food producers in process validation and shelf-life estimation.

[2] https://ec.europa.eu/food/safety/novel_food/legislation_en
https://ec.europa.eu/food/sites/food/files/safety/docs/fs_novel-food_leg_list_comp_auth_reg_2018_en.pdf
[3] https://ec.europa.eu/food/safety/novel_food_en

[4] Health Canada Position - High pressure processing (HPP) is no longer a novel process. www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/position-high-pressure-processing-no-longer-novel-process-treated-food-products-treated-food-products-2013.html [Last accessed on October 28, 2020].
[5] New Zealand Food Safety, Proposed update to guidance document on further processing: high-pressure processing. www.mpi.govt.nz/news-and-resources/consultations/proposed-update-to-guidance-document-on-further-processing-high-pressure-processing [Last accessed on October 28, 2020].
[6] Food Authority, NSW Government. Product considerations in HPP. www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/high_pressure_processing.pdf [Last accessed on October 28, 2020].

8 | SUSTAINABILITY ASPECTS OF HP TREATMENTS OF FOODS

So far, great progress was made for better understanding the basic principles of HHP, kinetics of microbial and enzyme inactivation, as well as its impact on food quality attributes and constituents (Barba et al., 2015; Hendrickx & Knorr, 2001; Knorr et al., 2011; Oey et al., 2008; Terefe et al., 2014). Despite significant numbers of machines operating in the industry already, HHP is still considered an emerging technology and food manufacturers currently using it, pioneers. Technological innovations and changes often come with economic and success risks, but at the same time, they can represent long-term perspective for sustainable development and competitive production. HHP offers a potential for improving products' quality and safety, but also has potential in generating value added products from agricultural crops. Considering the large amounts of energy and waste related to agriculture and food production (Monforti-Ferrario & Pascua, 2015), food waste reduction is of major importance for energy and improvement of environmental impact of the whole food chain. Finally, the application of HHP for shelf-life extension of seasonal products, without compromising product quality, would allow utilization of large, often excess amounts of products during the harvest period and their conversion into high-quality products with extended shelf-life (Aganovic et al., 2017).

One of the tools to assess environmental sustainability of different products, processing technologies, or services is certainly life cycle assessment (LCA), internationally standardized (ISO 14040, 2006; ISO 14044, 2006) and encouraged by the EU (EC, 2003) and governments around the world, or a more comprehensive life cycle sustainability analysis (Guinée et al., 2011). LCA distinguishes between different environmental impact categories, such as global warming or energy used, and assigns the environmental impacts to the different categories. Within each category, environmental impacts are assessed and calculated to equal units. LCA enables the estimation of direct and indirect environmental impacts that may occur along the supply chain of a product or technology and helps to identify opportunities to improve processes and resource use (Goedkoop et al., 2013; Goedkoop & Spriensma, 2001; Jolliet et al., 2003).

Compared to assessments of HHP and its impact on food quality and safety, where numerous studies are available (Sections 4 and 5), the number of studies on environmental aspects of HHP is rather scarce. To the best of the authors' knowledge, one of the first studies was performed by Pardo and Zufía (2012) for LCA of different technologies (autoclave pasteurization, microwaves, HHP, and modified atmosphere packaging) for production of a ready-to-eat meal based on fish and vegetables. The study indicated 15% lower environmental impact in cumulative energy demand for HHP compared to thermal treatment. It was concluded that alternative technologies may lead to reductions in environmental impact compared to traditional thermal processes. However, in this study, specific information on type and scale of technologies used, and their comparability is lacking. In the study of Davis et al. (2010), the environmental impact of PEF and HHP was compared to thermal pasteurization of carrot juice. It has been concluded that the energy used for pasteurization was relatively lower compared to total life cycle energy use, resulting in no significant differences between the selected technologies. A few later studies aiming on "a fair" basis comparison of HHP, PEF, HTST, microwave, and ohmic heating followed, considering the scale of production and industrial relevance (Aganovic et al., 2017; Atuonwu et al., 2020; Atuonwu et al., 2018; Cacace et al., 2020). These studies provided different outcomes for HHP technology mostly due to the differences in consideration of energy recovery for thermal process (Aganovic et al., 2017), or its exclusion from the equation (Atuonwu et al., 2018). The study of Aganovic et al. (2017) indicated no significant differences between HHP, PEF, and conventional thermal technologies in terms of energy consumption and environmental impact applied to watermelon and tomato juices at a pasteurization equivalent. A recent publication, however, indicates higher sustainability potential for ohmic heating and HHP technologies if energy recovery is not applied for conventional thermal technologies (Atuonwu et al., 2018). Theoretical model calculations of specific energy requirements for HHP, membrane filtration, PEF, ultraviolet radiation, and HTST confirmed relatively high energy demand for HHP (Rodriguez-Gonzalez et al., 2015). Cacace et al. (2020) reported that HHP is less expensive than MAP and also generates a lower environmental impact in almost all considered impact categories.

In conclusion, the environmental impact of HHP, as well as its potential direct social effect through nutrition, seems to be in the range of impacts of conventional thermal and other alternative technologies (PEF, microwave, and ohmic heating). Batch process, low filling ratio, and limitations in energy/pressure recovery are the major factors that could be improved to influence the relatively high environmental impact of food treated by HHP.

9 | RESEARCH NEEDS FOR HP APPLICATIONS

Despite significant empirical knowledge and the identification of general treatment conditions for at least some foods, there are still certain challenges associated with

application of HHP with regard to safety of products and expanded application range. Respective points of research interest are presented in Online Appendix Table 9.

From the *process engineering* perspective, ensuring process homogeneity and uniform temperature distribution during HHP treatment is of great importance. This is even more important for HPT processes and inactivation of spores. Thus, reliable detection of flow profiles and flow distribution along with temperature distribution within the HP vessel during pressure build up and holding time requires development of special temperature sensors. This is becoming increasingly important when considering the variety of high-pressure systems available, different dimensions and volumes of the HP vessel, in combination with varying pressure and temperature conditions and possible different pressure transmission media that can be potentially used (e.g., water, alcohol, oil, or mixtures), and finally variety of foods and their composition. From the perspective of mechanical engineering and material science, improvement of seals and valve technology, along with the design of the HP vessel, represents special challenges, especially for bulk-machines, where risk of recontamination seems to be higher compared to standard batch systems.

Despite significant amounts of available scientific data, the *inactivation of vegetative microbial cells* by HHP is still not fully understood. So far, for the specific food groups, there are no validated process control indicator microorganisms and no apathogenic surrogates for process validation studies. In terms of *spore inactivation* using pressure and temperature combinations, a rationale should be developed on the impact of food components on bacterial endospore inactivation. Apart from approaches using one substitute for each major food component class, the detailed composition of each of such classes and minor, specific components deserve attention. The spore germination mechanisms and cortex lytic machineries of different types of *C. botulinum*, *C. perfringens*, and *B. cereus* should be characterized at molecular level to derive approaches for the setup of targeted HPT processes toward their inactivation. Investigation of *virus inactivation* by HHP is limited due to lack of systems for direct infectivity measurement, especially for the food relevant human norovirus and hepatitis E virus. Inactivation of the latter one is of major importance for meat products. Therefore, the development of reliable cell culture systems for infectivity determination of these viruses is considered to be of high priority.

Reviewing *chemical reactions* and changes in food that take place under HHP, no safety-relevant issues could be identified to date. However, a key understanding of chemical reactions taking place under pressure and general statements about their course is missing. Regarding the MRs, further research should clarify the kinetics of Amadori product formation and degradation. In this context, the behavior of carbohydrate degradation or conversion, respectively, will be of importance and more data need to be obtained in this subject. An open issue is the quantitative relevance of arginine reactions. Above this, due to the complexity of food, it will be necessary to include the coaction of carbonyls, which originate from sources like lipid oxidation, which may be promoted by pressure. Finally, the processes above can have a significant impact on storage stability of HPT food and should be examined at larger scale. Very often, when investigating impact of HHP on food structure, the consequences of the caused cell disruption and adverse effects, especially during storage, are neglected.

Availability of conclusive data on food *allergenicity* under HHP, especially with regard to *in vivo* data, is still scarce. Investigations of allergenic potential should be extended to a broader range of foods and conducted in accordance with standards of allergy research. Possible aspects of neosensitization due to structural changes in proteins and allergens should be investigated in suitable cell and animal models.

So far, there are significant empirical data on suitability of different *packaging* materials for HHP. However, there are only limited data on mass transfer from packaging materials to food (migration) treated by HHP, behavior of polymers under HHP, and resulting changes in properties of the packaging material, especially with regard to the changes in the crystallinity of plastics. Studies on structure and morphology of packaging materials under pressure conditions, considering multi-layer structures and any migration phenomena that may occur, are still scarce. The behavior of packaging materials under HP should be systematically investigated for the migration of substances. These results are required for future design and optimization of packaging materials suitable for HHP applications.

10 | CONCLUSION

HHP treatment is a complex technology that has been used for preservation of fruit juices since the early 1990s, and over the course of years has undergone continuous development and application broadening for other food products. Based on practical experience and numerous studies, the conditions of a successful HHP application, where shelf-life comparable to that of pasteurization is achievable, can be named for individual food groups, such as fruit juices, fruit and vegetable preparations, and certain meat products. Exact pressure boundaries and critical values for temperature, pH, and a_w value that will still ensure safe products are difficult to indicate. The pressure

necessary for inactivation of viruses is in many instances slightly lower than that for vegetative bacterial cells; however, data are missing especially for the food relevant human virus types due to the lack of methods for their infectivity determination. Parasites can be inactivated by comparatively lower pressure than vegetative bacterial cells. Aiming on maintaining fresh characteristics and attributes of unprocessed or minimally processed products, HHP treatment around the boundaries of effectiveness can result in inadequate inactivation of pathogens. Under conditions typically used for food processing, the chemical changes are of limited extent. Despite possible shorter shelf-life due to lipid or protein oxidation, currently, no adverse effects have been reported. Nevertheless, a better chemical understanding of HHP is of relevance for process control and product development. Compared to conventional processes, HHP treatment reduces the allergenic potential to lesser extent. For allergic individuals, this can influence the selection of safe food, contrary to previous experience with tolerated food that was heated for example. In the HPT combination procedures for killing bacterial endospores, each food must be evaluated individually regarding the effectiveness of the treatment. The data available on the influence of HHP treatment on packaging materials are very limited.

AUTHOR CONTRIBUTIONS

All authors contributed to structure and drafting the manuscript, while Angelika Roth and Kemal Aganovic collected and organized the data and finalized the manuscript. The authors of the publication are listed in order of the sections they wrote. Niels Bandick, Sabine E. Kulling, Dietrich Knorr, Karl-Heinz Engel, and Volker Heinz critically revised the manuscript. All authors were involved in writing–original draft; writing–review and editing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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