# Chapter 21
# Reaction Chemistry at High Pressure and High Temperature

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**Abstract** Conventional thermal processing technologies, widely used to increase food safety and stabilize foods, can cause extensive chemical changes in foods. The food industry has been improving conventional and developing new technologies in response to consumers demand for high safety standards and close-to-fresh quality foods with high nutritional value. Pressure-assisted thermal processing (PATP), also called pressure-assisted sterilization (PATS) if the desired level of bacterial spore inactivation is achieved, is an alternative technology based on applying high temperature under high pressure. In the specific case of conduction-heating foods, adiabatic compression heating facilitates reaching temperatures lethal to microorganisms, which, in combination with fast decompression cooling, lowers quality degradation to levels below conventional thermal processing. However, its implementation requires advances in the analysis of reaction kinetics at high pressure and elevated temperature. Unfortunately, very few studies have focused on PATP effects on chemical reaction rates of quality factors at the temperature and pressure levels required for the sterilization of low-acid foods. Even fewer studies have focused on the effect of pressure on thermal degradation reactions known to form toxic compounds. At present, it is not possible to predict whether pressure will increase or decrease the rate for the degradation of quality factors and the formation of toxic compounds unless its activation volume value ($V_a$) is determined experimentally. Reactions with negligible rate under conventional pressure are of particular interest because they could become important in PATP-treated foods if their $V_a$ value is negative and large. Such reactions will be greatly accelerated by pressure and could

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constitute a significant cause of safety risk and quality degradation. The evaluation of the safety risk in PATP-treated foods is therefore necessary but this need is still largely ignored by funding agencies in the USA. For example, temperatures in excess of 120 °C are required for a detectable formation of acrylamide in foods treated conventionally, but if this reaction had a large negative $V_a$ value, significant levels could be formed at lower temperatures and high pressure. Although a recent model solution study conducted in Europe demonstrated that acrylamide formation is actually inhibited by pressure, this favorable finding must be confirmed with experiments in actual foods.

**Keywords**  High-pressure processing • Reaction chemistry • Activation volume

## 21.1   Introduction

A significant recent development in the food processing industry has been the commercialization of high-pressure processing (HPP) at room or refrigeration temperature. However, HPP provides only a pasteurization effect since the inactivation of bacterial spores by pressure alone is not feasible (Torres et al. 2010; Torres and Velazquez 2008; Mújica-Paz et al. 2011; Serment-Moreno et al. 2015). An emerging and particularly innovative conservation technology, pressure-assisted thermal processing (PATP) or pressure-assisted thermal sterilization (PATS), could facilitate meeting the demand for more healthy, nutritious, varied, and convenient processed foods. This development is based on the combined application of high pressure and high temperature, typically in excess of 600 MPa and 100 °C (Shao et al. 2010; Valdez-Fragoso et al. 2011; Serment-Moreno et al. 2014). PATP promises to yield shelf-stable foods of high quality and reduce the loss of constituents with desirable health benefits (Torres et al. 2009b; Pérez Lamela and Torres 2008a, b; Escobedo-Avellaneda et al. 2011). However, PATP processing conditions can break covalent bonds and thus losses of nutrients, color pigments, and flavor compounds should be expected. Furthermore, under PATP conditions, the formation of some toxic compounds may reach rates not observed in conventional thermal processes (Segovia-Bravo et al. 2012). Therefore, the severity of PATP conditions achieving the inactivation of bacterial spores and baroresistant enzymes must be approached carefully to meet microbial safety and shelf-life goals. Unfortunately, very few comprehensive reports have been published on PATP effects on the kinetics of chemical changes in foods (e.g., Ramirez et al. 2009; Saldaña and Martinez-Monteagudo, 2014).

Typical HPP processing conditions include pressure levels of 500–600 MPa, initial temperature below 40 °C, and a holding time of 1–5 min. HPP products currently in the market include refrigerated products such as fruit juices, deli meats, seafood, ready-to-eat meals, and salsas (Torres et al. 2009a; Torres and Velazquez 2008; Ulloa-Fuentes et al. 2008a, b). Consumer perceptions that HPP products are closer to "natural" and have a high retention of nutrients including health-enhancing

components with high market value have facilitated the market acceptance of these products (Cruz et al. 2011). On the other hand, PATP is not yet a commercial technology and will require more complex safety validation procedures than HPP, particularly for the production of shelf-stable low-acid foods (pH > 4.5). However, there is an important commercial interest in this new process alternative as reflected by the availability of commercial 35–55 L PATP prototypes being used for research on process and product development in Europe and the USA. In PATP processing, foods are first preheated to 70–90 °C, after which the temperature increases to levels lethal to bacterial spores due to the adiabatic heating of the food and the pressurizing fluid upon pressurization. Subsequent decompression reduces product temperature to values below those causing significant thermal degradation. In addition, it is possible to identify pressure and temperature combinations accelerating spore inactivation rate. These two effects reduce the severity of thermal treatments, particularly for conduction-heating foods, resulting in large quality improvements without compromising food safety.

The analysis of chemical reactions in PATP-treated foods is necessary when marketing foods in countries regulated by novel food laws. In the European Union (EU), novel foods were originally defined as foods and food ingredients not used for human consumption to a significant degree prior to May 1997 (Hepburn et al. 2008). In the EU and other countries following similar regulations (e.g., Canada), the safety evaluation of PATP-treated foods must follow the comparative principle of "substantial equivalence," i.e., they must be compared with comparable products obtained by conventional technologies. If undesirable compounds are detected in the PATP product, a detailed risk assessment of these compounds must be carried out including hazard identification, characterization, and evaluation of the consumer exposure (Tritscher 2004). By contrast, in the USA, a PATP sterilization process approved for mashed potatoes required no such characterization of toxicity risk (Anonymous 2009).

## 21.2 Effect of Extrinsic and Intrinsic Factors on Chemical Reactions under High Pressure

Information on the retention of vitamins, pigments, and flavor compounds under PATP conditions ensuring microbial and enzyme inactivation will require substantially more research. Although the maximum pressure covered in published studies on chemical changes in foods and model systems under PATP conditions is ~600–850 MPa, temperatures above 100 °C required to achieve the sterilization of low-acid foods are rarely included. Also, studies are needed to evaluate the effect of dissolved oxygen on the chemical degradation of flavor compounds and nutrients in PATP-treated foods since Oey et al. (2006) demonstrated that PATP degradation rates can increase with the dissolved oxygen concentration.

Serment-Moreno et al. (2014) reviewed recently a very large number of primary and secondary kinetic models available to design PATP treatments. However, the reliability of values predicted by these models will depend also on the availability

and quality of the experimental data. Unfortunately, missing data such as sample temperature, pressurization rate, insufficient sample characterization including dissolved oxygen concentration and at least initial and posttreatment pH which has not been measured while these foods are under high pressure and high temperature, limitations of applying gas reaction chemistry to chemical reactions under PATP conditions, and incorrect applications of physical principles can be found in publications on high-pressure research. For example, the Le Chatelier principle predicts a displacement of the equilibrium point for chemical reactions and is associated with the reaction molar volume change $\Delta V$, defined as the difference between the partial molar volume of products and reactants. This value can be estimated from the partial derivative with respect to pressure of the reaction equilibrium constant $K$ (Torres et al. 2009b, 2010):

$$V = -RT(\frac{\partial \ln K}{\partial p})_T \tag{21.1}$$

One of the most important aspects frequently overlooked in the analysis of PATP treatments is the pressure-induced pH change. Water ionization changes under high temperature-high pressure resulting in a pH decrease. Marshall and Franck (1981) estimated the water ionization constant ($-\log K_w$) and predicted a drop of 1.09–1.46 pH units when water is pressurized in the 400–700 MPa and 70–100 °C range. Even though during depressurization pH might return to its original value, the pressure-induced pH shift while the food is under high pressure will affect the rate of chemical reactions and the inactivation rate of enzymes and microorganisms (Paredes-Sabja et al. 2007). Interpreting pH effects in food systems under pressure is challenging because the parameters to predict the pressure-induced pH shift (Eq. 21.2, El'Yanov and Hamann 1975; Neuman et al. 1973) have not been determined for foods. However, the reaction molar volume change for organic acids and buffers frequently found in biological system has been determined by spectrophotometric methods. Anionic solutions are usually accompanied by a negative change, whereas cationic and zwitterionic solutions show a slight pH increase (Gayán et al. 2013; Hayert et al. 1999; Kunugi 1992; El'yanov and Hamann 1975; Neuman et al. 1973):

$$(pK_a)_p = (pK_a)_0 + \frac{p(\ V^0)}{RT(1+bp)} \tag{21.2}$$

where:

$(pK_a)_p$ = pressure-shifted dissociation constant
$(pK_a)_0$ = dissociation constant at the reference pressure (0.1 MPa)
$p$ = pressure (MPa)
$\Delta V^o$ = reaction molar volume change $\Delta V$ of the dissociating acid at 0.1 MPa (m³/mol)
$R$ = universal gas constant, 8.31446 (Pa m³)/(K mol)
$T$ = absolute temperature (K)
$b$ = 9.2 × 10⁻⁴ MPa (assumed constant for all acids)

The limited consideration in published works of the food pH changes induced by pressure can be justified by the lack of practical and widely available instruments to
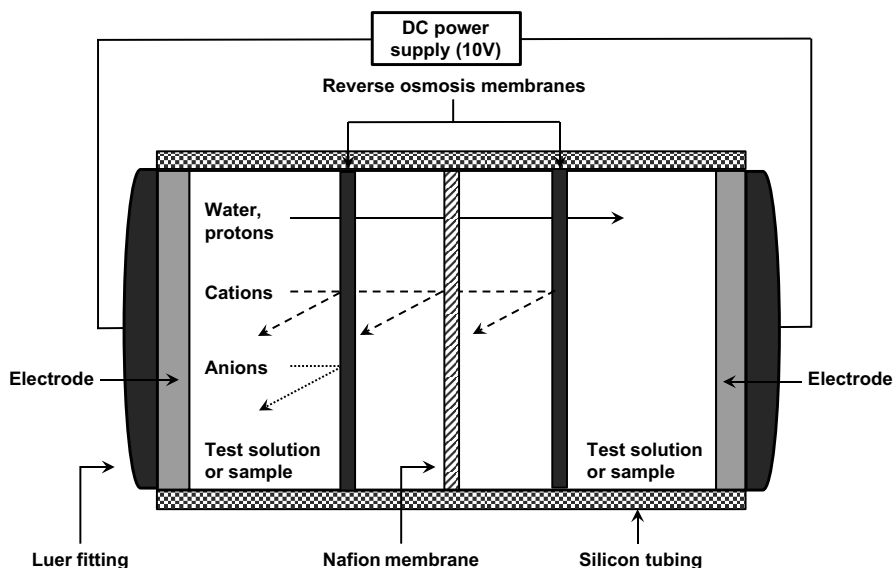
**Fig. 21.1** Schematic view of a pH sensor capable of measuring pH of semiliquid and liquid food samples up to 800 MPa and 25 °C. Modified from Samaranayake and Sastry (2013)

measure them by direct methods. Although Samaranayake and Sastry (2010, 2013) developed recently an in situ pH sensor for use at pressures up to 825 MPa at room temperature (25 °C), the device needs to be further validated and tested at high temperature. The pH sensor consists of a Nafion membrane placed between two reverse osmosis membranes that selectively isolate the movement of hydrogen ions from the test solution when a 10 V (DC) voltage is applied to the two chromel wires acting as electrodes (Fig. 21.1). The membrane assembly and test solution are enclosed in silicon tubing allowing pressure transmission and sealed with luer fittings. The electrical connections are insulated with a Teflon tube sealed with epoxy resin. The test solutions consisted of a buffer (0.05 M biphthalate at pH 4.01 or 0.025 M phosphate at pH 6.01) and diluted HCl (0.1 M at pH = 1.35) mixture used to correct pH measurements since its ion concentration is known considering that HCl is fully dissociated in water. Finally, the pressurized solution pH was modeled (Eq. 21.3) by relating the density ($\rho$) and proton conductivity ($\sigma^+$) ratios at atmospheric pressure condition taken as a reference (subindex 0) (Samaranayake and Sastry 2008, 2010):

$$(\text{pH})_0 - (\text{pH})_P = \log(\rho_P / \rho_0) + \log(\sigma_P^+ / \sigma_0^+) \tag{21.3}$$

The pH sensor developed by Samaranayake and Sastry (2010) has been used to determine the pressure-induced pH shift in buffer systems and in semiliquid and liquid foods at 0.1–785 and 0.1–800 MPa, respectively (Samaranayake and Sastry 2013). The pH of organic (acetate, biphthalate, phosphate, and sulfanilate) and biological (ACES, citrate, HEPES, MES, and TRIS) buffers decreased by 0.05–0.32 units at 785 MPa ($p < 0.05$), although the acidity of the solutions remained fairly

**Table 21.1** Pressure effect on the pH of commercial food products (adapted from Samaranayake and Sastry 2013)

| Food sample | $pH_0$ (0.1 MPa) | pH (800 MPa) | Steepest pH decline (Pressure range) |
|---|---|---|---|
| *Liquids* | | | |
| Deionized water | 5.50 | 4.81 | −0.69 ± 0.07 (0.1–800 MPa) |
| Fruit juices (apple, grapefruit, orange, tomato) | 3.30–4.30 | 3.00–4.00 | <0.3 (100–500 MPa) |
| Milk | 6.60 | 6.30 | <0.3 (100–500 MPa) |
| Chicken broth (99 % fat-free) | 5.80 | 5.19 | −0.37 ± 0.02 (0.1–100 MPa) |
| *Semiliquids* | | | |
| Guacamole | 3.90 | 3.50 | −(0.3–0.4) (0.1–100 MPa) |
| Ranch dressing | 3.35 | 2.95 | −(0.3–0.4) (0.1–300 MPa) |
| Yogurt (nonfat) | 4.25 | 3.61 | −0.58 (0.1–300 MPa) |

constant as no significant differences were observed for pressures higher than 588 MPa. Nevertheless, the initial pH of all buffers was restored after depressurization indicating that the acidity increase was pressure driven. The values observed for organic buffers (Eq. 21.3) were compared to the pH predictions obtained with Eq. (21.2). Differences were more evident as the pressure level increased and the authors attributed the discrepancies to the methodology approach for pH measurement (optical methods vs. pH sensor) used on each study (Samaranayake and Sastry 2010). The pH changes of distilled water and several commercial food products measured by Samaranayake and Sastry (2013) are shown in Table 21.1. Distilled water experienced the highest pH decrease (–0.69 ± 0.07 units). Milk and fruit juices exhibited a slight pH change (<0.3 units) up to 500 MPa as previously described for buffer solutions (Samaranayake and Sastry 2010). The pH evolution of pressurized chicken broth and semiliquid foods was similar, showing a higher acidification at low pressure range (100–300 MPa) before stabilizing at 0.30–0.58 pH units below their initial value. The authors suggested that the stability of milk and fruit juices reflected the presence of weak organic acids in food which act as buffer agents reducing the impact of pH change. In the case of chicken broth and semiliquid foods, Samaranayake and Sastry (2013) proposed that the high compressibility of fats and enclosed air bubbles favored hydrogen bond formation that resulted in a steep pH fall below 300 MPa, whereas the breakdown of hydrogen bonds and the exposure of hydrophobic moieties of denaturalized proteins led to the subsequent pH stabilization at 300–800 MPa. Although the measurements performed by the pH sensor for both buffers and food products are certainly promising, Samaranayake and Sastry (2010, 2013) concluded that further experimentation is needed to reach an agreement with previous research and to elucidate ionization mechanisms under high pressure.

## 21.3 Applications to Dairy Flavor Volatiles

Changes in the rate of chemical reactions during food processing are more important than the equilibrium point shift predicted by the Le Chatelier principle because the processing time is too short to reach it. A primary kinetics model describing the changes with time ($t$) in concentration ($c(t)$) as affected by pressure ($p$) and temperature ($T$) for chemical reactions of any order $n$ can be expressed as follows:

$$\frac{d\,c(t)}{d\,t} = k(p,T)\,c(t)^n \tag{21.4}$$

where $k$ is the reaction rate constant at a given pressure and temperature. Under isobaric and isothermal conditions, integration of Eq. (21.4) yields the following expressions:

$$\text{Zero order}: \ c(t) - c_0 = k(p,T)t \tag{21.5}$$

$$\text{First order}: \ \log c(t) - \log c_0 = k(p,T)t \tag{21.6}$$

$$\text{Second order}: \ \frac{1}{c(t)} - \frac{1}{c_0} = k(p,T)t \tag{21.7}$$

The expression with the best correlation coefficient ($R^2$) for experimental determinations of $c(t)$ conducted at several "constant" $p$ and $T$ levels is then used to determine pressure and temperature effects on the rate constant $k$. A major challenge is to obtain experimental data at constant $p$ and $T$ levels starting from food initially at atmospheric pressure and at refrigeration or room temperature considering that the pressure vessel, pressurizing fluid and the food will need to be preheated to reach lethal temperatures to the microorganism of concern. Differences in thermophysical properties among food components and the pressurizing fluid, combined with heat transfer to the pressure vessel and the environment, result in significant food temperature changes with time and placement in the vessel. If the temperature is not constant and not uniform, this will impact the observed rate of the chemical reactions and microbial or enzymatic inactivation. These rate expressions must be taken into account in process design and optimization (Grauwet et al. 2010a, b, c, 2011, 2012; Khurana and Karwe 2009; Rauh et al. 2009; Torres et al. 2009b). This also means that the $c_o$ value in Eqs. (21.5)–(21.7) is at best a "pseudo-initial concentration." If $c_o$ values at each test temperature do not change with pressure regardless of the treatment time, it will confirm that sample handling effects and heat losses have been minimized.

The theoretical frame developed originally for gas reactions (Serment et al., 2014) postulates that reactants must reach first an activated state (Fig. 21.2a) characterized by the temperature-independent Arrhenius activation energy ($E_a$) value estimated at constant pressures using Eq. (21.8). The slope of this curve is $-E_a/R$
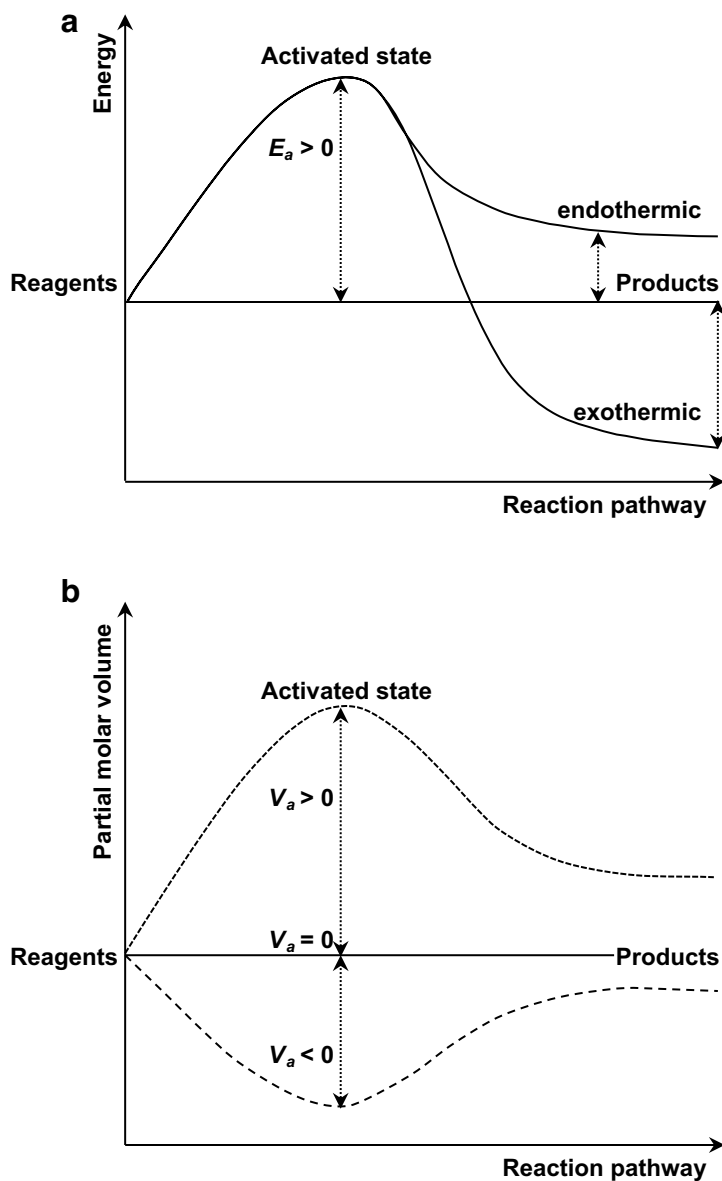
**Fig. 21.2** Definition of the activation energy ($E_a$) for endothermic and exothermic reactions (**a**) and activation volume ($V_a$) for reactions inhibited ($V_a>0$), inhibited ($V_a<0$), or not affected by pressure ($V_a=0$) (**b**)

($R$=universal gas constant, $8.314 \times 10^{-3}$ kJ mol$^{-1}$ K$^{-1}$) and the intercept is ln $k_o$ ($k_o$=pre-exponential constant):

$$\ln(k) = \ln(k_0) - \frac{E_a}{RT} \qquad (21.8)$$

In the case of PATP-treated foods, the pressure effect on $E_a$ values is analyzed by introducing the *partial activation molar volume* ($V_a$) concept (Fig. 21.2b), defined as the difference of the partial molar volume between the activated state and the unreacted molecule at the same pressure and temperature (Eq. 21.9, McNaught and Wilkinson 1997). Equation (21.9) can be integrated to obtain Eq. (21.10) where ln $A$ is the integration constant. Values for $V_a$ at constant temperature are obtained by linear regression of ln($k$) versus pressure $p$:

$$V_a = -RT(\frac{\partial \ln k}{\partial p})_T \tag{21.9}$$

$$\ln k = \ln A - \frac{(V_a)\ p}{R\ T} \tag{21.10}$$

$E_a$ values required to reach the activated state are always positive while $V_a$ values may be negative, positive, or zero (Torres et al. 2009b). The sensitivity of a chemical reaction rate to pressure depends on the absolute value of $V_a$ (positive or negative). Large-magnitude $V_a$ values (positive or negative) mean that the chemical reaction is highly sensitive to pressure while reactions with $V_a = 0$ are pressure independent. The activation energy $E_a$ will decrease, remain unchanged, or increase with pressure if $V_a$ is negative, zero, or positive, respectively. At constant temperature, the rate of a reaction will increase with pressure if $V_a$ is negative while the opposite effect will be observed if $V_a$ is positive. When $V_a$ values are negative, the reaction kinetic constant $k$ at constant temperature will increase with pressure. The opposite behavior will be observed for reactions with $E_a$ values increasing with pressure, while no pressure effects on $E_a$ values will correspond to reactions with $V_a = 0$. Chemical reactions with $V_a \gg 0$ will be so slow at high pressure that no changes will be observed during the PATP-treatment time needed to inactivate enzymes and microorganisms. This will result in a much enhanced quality of PATP-treated foods. On the other hand, in reactions with a very large negative $V_a$, the concern will be a significant loss of quality (e.g., loss of nutrients) and a higher formation of undesirable compounds.

The approach previously described was used to analyze the formation of 27 volatile compounds associated with cooked flavor in PATP-treated milk (Vazquez et al. 2007). In the case of straight-chain aldehydes, first-order kinetic constants fitted well the Arrhenius model with activation energy ($E_a$) values decreasing significantly with pressure. For example, $E_a$ values for hexanal formation decreased nearly 40 times from 35.2 kJ mol$^{-1}$ at 482 MPa to 0.9 kJ mol$^{-1}$ at 655 MPa. In reactions following zero-order kinetics, $E_a$ values increased with pressure for 2-methylpropanal and 2,3-butanedione but remained practically unchanged for hydrogen sulfide regardless of the pressure level. Most significantly, the concentration of the remaining 18 volatiles analyzed in PATP milk did not change under high pressure, suggesting that their formation reactions had $V_a \gg 0$. These findings provided an explanation to a previous principal component analysis (PCA) indicating that the volatile profiles

of PATP- and conventionally pasteurized milk are significantly different (Vazquez 2006; Vazquez et al. 2006). PCA showed that PATP milk treated at moderate temperature and pressure levels with a refrigerated shelf life longer than 7 weeks had a volatile profile similar to thermally pasteurized milk with a shelf life of only 2–3 weeks. A further observation was that for severe PATP processes, i.e., applying temperature conditions approaching those needed for commercial milk sterilization in combination with pressure, the PCA showed a smaller and different-direction volatile profile shift to the one observed for commercial UHT milk. Both findings are consistent with the observation that pressure had a different effect on the formation of the 27 volatile compounds analyzed in PATP milk. For example, aldehyde formation was accelerated while the reactions leading to the formation of 18 compounds were inhibited to negligible rates. Another important conclusion from the PATP milk study is that the increase, decrease, or lack of change caused by pressure and temperature on the formation of volatiles in PATP milk can be described with no need to assume alternative reaction pathways.

## 21.4   Assessment of a Potential Chemical Toxic Risk in PATP-Treated Foods

The benefits of heat treatments as a preservation method and to improve desirable food qualities such as palatability and nutritional value are well established. However, the formation of undesirable compounds is also possible, and subsequently, chemical toxic risks are receiving considerably more attention even in conventionally processed foods, particularly in Europe (e.g., Eisenbrand et al. 2007). The assessment of high hydrostatic pressure processing of foods at high temperature must consider potential chemical toxicology risks in addition to ensuring microbial safety, product stabilization, and the retention of flavor compounds, nutrients, and functional ingredients. These risks depend greatly on the presence of molecules with known influence in the potential formation of toxic compounds (e.g., reducing sugars and free amino acids via the Maillard reaction). The health hazards of Maillard reaction compounds such as acrylamide and of many other chemical compounds formed during thermal processing (e.g., heterocyclic aromatic amines, furans, and monochloropropanediol) should be evaluated in foods subjected to PATP treatments. Other reactions occurring during the heating of foods, such as the oxidation of unsaturated fatty acids and the advanced glycation between amino acids and sugars, have undesirable nutritional and health effects, and they must be considered also when treating high-fat products with PATP (Kanekanian 2010).

The next sections will cover examples of chemical reactions responsible for toxic risks in foods and the very few studies analyzing them under PATP conditions (Escobedo-Avellaneda et al. 2011; Segovia-Bravo et al. 2012). Unfortunately, the kinetics of formation of acrylamide, polycyclic aromatic hydrocarbons, heterocyclic amines, N-nitroso compounds, and other chemicals known for their toxicological risks in thermal processing is mostly unknown under PATP conditions.

Although theoretical considerations indicate that chemical reactions involving positive $V_a$ values will be inhibited by high pressure and lead to a decreased formation of undesired compounds and/or significantly lower nutrient losses than under conventional thermal processing, it is not yet possible to predict whether the $Va$ value will be positive or negative. Therefore, demonstrating the absence of undesirable chemical changes in PATP-treated products requires experimental evidence. On the other hand, chemical changes examined so far in PATP-treated foods and model systems have followed known reaction mechanisms making it unnecessary to postulate new reaction mechanisms. Finally, it is important to highlight that at present there is *no experimental* evidence questioning the safety of PATP and HPP foods. Moreover, some studies have shown a toxicity reduction such as a decrease in the formation of biogenic amines in ripened meat products treated by HPP (Ruiz-Capillas et al. 2007; Ruiz-Capillas and Jiménez Colmenero 2004) and an inhibition of acrylamide formation in model systems subjected to PATP treatments (de Vleeschouwer et al. 2011).

### 21.4.1  *Acrylamide Risk*

The Maillard reaction between amines and carbonyl compounds, which generates pleasant flavor and color compounds in cooked foods, can lead to acrylamide formation during the heating of foods containing the amino acid asparagine. In the first step of the reaction, asparagine reacts with a reducing sugar forming a Schiff's base which follows a complex reaction pathway including decarboxylation and a multistage elimination reaction (Zyzak et al. 2003). Acrylamide formation investigated in model systems has shown that free asparagine is a limiting factor since asparaginase treatment can prevent its formation (Weisshaar 2004). The formation of minor amounts of acrylamide in the presence of glutamine and methionine has also been reported (Stadler et al. 2002). In 2002, the Swedish National Food Authority and the University of Stockholm reported finding considerable acrylamide levels in starch-based foods (Anonymous 2002). Since acrylamide is neurotoxic, induces germ cell mutagenicity, and is classified as probably carcinogenic to humans, finding it in foods had a major international impact. Later research efforts have focused on confirmations of initial findings (Matthäus 2004; Zyzak et al. 2003), mechanisms of formation (Zhang and Zhang 2007; Zyzak et al. 2003), improvements to analytical methods (Kim et al. 2007), and efforts to reduce its level in processed food (Haase 2004). The concerns about acrylamide presence in foods reflect several evidences of safety risks. Glycidamide, an acrylamide metabolite that binds to DNA causing genetic damage, has been found in studies with mice, rats, and humans exposed to acrylamide. In vitro and in vivo studies have shown that acrylamide induces gene mutations in cell cultures and in animal studies (Gamboa da Costa et al. 2003). Neurological damage was observed when rats were given acrylamide in their drinking water and also in humans exposed to high acrylamide doses (Fullerton and Barnes 1966). Finally, decreased fertility has been observed in rats exposed to

5–10 mg acrylamide/kg body weight per day (Anonymous 2002). Under conventional thermal processing, significant acrylamide formation has been demonstrated to require temperatures higher than 120 °C (Pedrenski 2007). If the acrylamide reaction pathway were characterized by a large negative $V_a$ value, pressure would accelerate its formation and significant levels could be formed even in foods processed at lower temperature but high pressure. The pressure-induced pH shift during PATP treatments also could affect the amount of acrylamide formed.

The acrylamide reaction has been under investigation for some time (Claeys et al. 2005a, b; de Vleeschouwer et al. 2006, 2008a, b; Anonymous 1994; Weisshaar and Gutsche 2002). The Maillard reaction is affected by high pressure and different effects have been reported, i.e., increasing or decreasing the formation of intermediate or final products depending on the reaction stage evaluated (Moreno et al. 2003; Isaacs and Coulson 1996; Schwarzenbolz et al. 2000, 2002) which increased concerns about the lack of studies on the acrylamide formation in PATP-treated foods. In model systems, de Vleeschouwer et al. (2011) showed that the amount of acrylamide formed at 115 °C and 600 MPa is much lower than the one observed for conventional thermal treatments (1700 ppb vs. 6500 ppb). The inhibition of acrylamide by pressure suggesting a positive $V_a$ value for the reaction needs to be confirmed in food systems.

## 21.4.2 Polycyclic Aromatic Hydrocarbons, Heterocyclic Amines, N-Nitroso Compound Risk, and Hormone-Like Peptides

The formation in foods of polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds with two or more fused aromatic rings, depends on the cooking method. Grilling meat, fish, or other foods with intense heat over direct flame is one of the cooking procedures increasing the formation of PAHs. At temperatures in the 400–1000 °C range, organic compounds are fragmented into smaller compounds yielding relatively stable PAHs. Benzo[α]pyrene, a five-ring polycyclic aromatic hydrocarbon, is the PAH most commonly studied because of its mutagenic activity being the most carcinogenic compound of all PAHs (Anonymous 1983; Lee et al. 1981). Benzo[α]pyrene, benz[α]anthracene, and dibenz[α,β]anthracene have been reported to be carcinogenic in animals by oral intake and considered as *probably* carcinogenic to humans. It remains to be determined if the lower temperatures to be used in the production of PATP-treated foods (typically under 120 °C) will result in significant formation of PAHs.

Another group of toxic compounds formed during food heating is heterocyclic amines grouped into amino-carbolines, imidazoquinolines, imidazoquinoxalines, and imidazopyridines and characterized by two or three rings with an exocyclic amino group. They are produced via the Maillard reaction from creatine or creatinine, certain free amino acids, and sugars when cooking or heating meat or fish (Jägerstad and Skog 2005; Jagerstad et al. 1998). If these three precursor groups are

present in a PATP-treated food, it should be analyzed for the presence of heterocyclic amines. In addition, foods treated by PATP containing nitrites, typically added to cured meat and fish products for *Clostridium botulinum* control, should be tested for the presence of genotoxic N-nitroso compounds. As there is no evidence that PATP affects their formation rate, PATP treatment of packaged foods opens the possibility of eliminating this additive.

Research has been focused also on other undesirable reactions that can take place under high pressure such as the formation of peptides with hormone-like effects. Chemical cyclization reactions could take place under high pressure and change the relative concentration of short-chain peptides in foods with unpredictable biological effects. For instance, Fernández García et al. (2003) reported that high pressure induces the formation of hormone-like substances by cyclization of glutamine at the N-terminus of certain peptides. The same authors have reported that application of pressure accelerates the formation of diketopiperazines, a peptide with biological activity formed from aspartame (Butz et al. 1997, 2002).

## 21.5 Screening the Formation of Mutagenic Agents in PATP-Treated Food

The identification of genotoxic substances is an important procedure in the assessment of processed food safety including novel foods. The Ames *Salmonella*/microsome mutagenicity assay is a rapid bacterial reverse mutation assay designed to detect a wide range of chemical substances that can produce genetic damage leading to gene mutations (Mortelmans and Errol 2000). In the test, several histidine-dependent *Salmonella* strains with different mutation genes of the histidine operon are used. These mutations are locations responding to the food compound to be tested. Some carcinogenic substances, such as aromatic amines and polycyclic aromatic hydrocarbons, are biologically inactive if they are not metabolized to active forms. In humans, these compounds are metabolized by the cytochrome-based P450 oxidation system in the liver; however, as bacteria do not have this metabolic system, a rat liver extract is added to the mixture of bacteria to activate the potential carcinogenetic chemical (Ames et al. 1973). In the test, *Salmonella* strains are grown in a media with very low levels of histidine, so only bacteria able to revert to an histidine-independent mutant can grow in that media and form colonies. The test is positive if the number of colonies that grow in the media without histidine is higher than the number of spontaneous revertants in media without the test substance. The difference is proportional to the dose effect of the test substance.

The single cell gel electrophoresis assay, also known as the comet assay, is a sensitive technique for detecting DNA damage at the level of the individual eukaryotic cell. This technique was first developed to detect DNA damage at the single cell level (Östling and Johanson 1984) and later the technique was modified with an alkaline electrophoresis step (Singh et al. 1988). The standard alkaline comet assay and its various modifications provide a relatively simple, sensitive, and rapid

method of analyzing DNA damage and repair. It is economical, simple, and fast, so its use has been rapidly expanding in recent years. It involves the encapsulation of cells in a low-melting-point agarose suspension. A sample of cells derived from an in vitro cell culture is dispersed into individual cells and suspended in molten low-melting-point agarose at 37 °C. This mono-suspension is cast on a microscope slide of cells lysed in neutral or alkaline (pH > 13) conditions followed by electrophoresis of the suspended lysed cells. The migrating DNA is quantified by staining with ethidium bromide and by measuring the intensity of fluorescence at two fixed positions within the migration pattern using a microscope photometer equipped with imaging software.

## 21.6   Conclusion

Although PATP products are not yet on the market, a near-future commercialization of this technology is expected since commercial prototypes are being now used to evaluate the quality and safety of PATP-treated foods at the laboratory and pilot plant level. Among the challenges to overcome, the unpredictable effect of pressure on the kinetics of chemical reactions at the high temperatures required to produce shelf-stable foods remains as one of the most important. This effort must ensure the absence of toxicological risks in PATP-treated foods through reaction studies like the ones here described, to determine whether the formation of these known unwanted compounds is accelerated or decelerated by PATP treatments. It may be also necessary to consider the possibility of new toxicological risks since the formation of compounds that are not present after conventional thermal processing at a detectable rate could occur in PATP-treated foods, reflecting chemical reactions with a large positive activation volume.

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