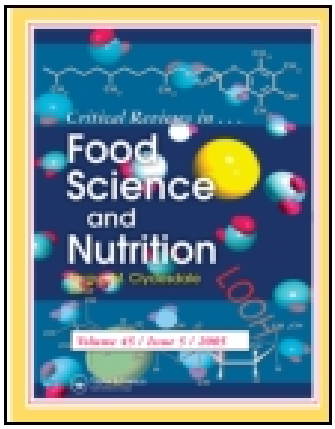

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Quality-Related Enzymes in Fruit and Vegetable Products: Effects of Novel Food Processing Technologies, Part 1: High-Pressure Processing

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Quality-Related Enzymes in Fruit and Vegetable Products: Effects of Novel Food Processing Technologies, Part 1: High-Pressure Processing

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The activity of endogenous deteriorative enzymes together with microbial growth (with associated enzymatic activity) and/or other non-enzymatic (usually oxidative) reactions considerably shorten the shelf life of fruits and vegetable products. Thermal processing is commonly used by the food industry for enzyme and microbial inactivation and is generally effective in this regard. However, thermal processing may cause undesirable changes in product's sensory as well as nutritional attributes. Over the last 20 years, there has been a great deal of interest shown by both the food industry and academia in exploring alternative food processing technologies that use minimal heat and/or preservatives. One of the technologies that have been investigated in this context is high-pressure processing (HPP). This review deals with HPP focusing on its effectiveness for controlling quality-degrading enzymes in horticultural products. The scientific literature on the effects of HPP on plant enzymes, mechanism of action, and intrinsic and extrinsic factors that influence the effectiveness of HPP for controlling plant enzymes is critically reviewed. HPP inactivates vegetative microbial cells at ambient temperature conditions, resulting in a very high retention of the nutritional and sensory characteristics of the fresh product. Enzymes such as polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME) are highly resistant to HPP and are at most partially inactivated under commercially feasible conditions, although their sensitivity towards pressure depends on their origin as well as their environment. Polygalacturonase (PG) and lipoxygenase (LOX) on the other hand are relatively more pressure sensitive and can be substantially inactivated by HPP at commercially feasible conditions. The retention and activation of enzymes such as PME by HPP can be beneficially used for improving the texture and other quality attributes of processed horticultural products as well as for creating novel structures that are not feasible with thermal processing.

Keywords High-pressure processing, pectin methylesterase (PME), polygalacturonase (PG), polyphenol oxidase (PPO), peroxidase (POD), β-glucosidase, chlorophyllase, allinase, lipoxygenase (LOX), cystine lyase, myrosinase, texture, consistency, flavor, color, enzyme inactivation, enzyme activation

INTRODUCTION

Enzymes are biocatalysts that are essential in the physiology and metabolism of plants. However, most enzymes remain active postharvest. Although this may be desired in cases where ripening takes place during postharvest storage, it may also lead to detrimental changes in quality attributes such as color, flavor, texture, and nutritional value. The activity of endogenous deteriorative enzymes together with growth of microorganisms (with associated enzymatic activity) and/or other non-enzymatic (usually oxidative) reactions considerably shorten the shelf life of horticultural products. Polyphenol oxidase (PPO), chlorophyllase, peroxidase (POD), lipoxygenase (LOX), and lipase may be responsible for color and flavor changes. Pectinases, cellulase, and hemicellulase may cause textural degradation. Thiaminase and oxidative enzymes such as ascorbic acid oxidase, PPO, and POD may cause loss of nutritional value (Whitaker, 1991). On the other hand, enzymes are used as catalysts or processing aids in many food-processing applications, usually to hydrolyze complex or large moieties into simpler molecules. There is also a growing interest to mimic nature in using enzymes to synthesize and transform molecules and improve the nutritional and

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organoleptic quality of food materials. Recent studies (Aguedo et al., 2004; Nemeth et al., 2004; Santiago-Gómez et al., 2007) on the application of soybean LOX and hydroperoxide lyase (HPL) to produce C6 aldehydes (the compounds responsible for "green" flavor of fresh vegetables) mimicking nature's "LOX pathway" is one such example.

The key enzyme responsible for a specific quality loss may vary from one product to another. For instance, LOX is the key enzyme in the development of off-flavor in green peas, green beans, and corn, while cystine lyase is the key enzyme responsible for the development of off-flavor in broccoli (with some contribution from lipase) and cauliflower (Whitaker, 1991). The main enzymes responsible for quality degradation may also be of microbial origin. For instance, the continued softening of apricots that is sometimes observed in canned apricot products has been shown to be due to a heat-stable polygalacturonase (PG) from a brown-rot fungus, while thinning of starch-based sauces packaged with green beans and green peas sometimes results from the activity of heat-stable amylases derived from microorganisms on the surface of the vegetables (Luh et al., 1978; Whitaker, 1991). Thermal treatment is the most common method of enzyme and microbial inactivation used by the food industry. Although generally effective in microbial and enzyme inactivation, thermal treatment may cause undesirable changes in product quality as well as nutritional value.

Increased awareness by consumers of the relationship between diet and health in recent years has created a greater impetus and effort for the exploration of alternative food processing technologies, which use minimal heat and preservatives with the aim of obtaining safe and fresh-like products of superior nutritional quality and acceptable shelf life. In this regard, novel technologies such as high-pressure processing (HPP), pulsed electric field, ultrasonication, UV irradiation, and alternative thermal-processing technologies such as microwave, radio frequency, and ohmic heating are being investigated. This review is Part 1 of a series of reviews that deals with the application of the major novel technologies in food preservation focusing on their effectiveness for controlling quality-degrading enzymes in fruit and vegatble products, which will simply be referred to as plant enzymes in most of the review. Part 1 deals with HPP.

QUALITY-RELATED ENZYMES IN FRUIT AND VEGETABLE PRODUCTS

Enzymes Related to Texture and Consistency

Texture is an important quality attribute of food products. The structural integrity and texture of fruits and vegetables can be attributed mainly to the primary cell wall, the middle lamella, and the turgor generated within cells by osmosis (Jackman and Stanley, 1995). Depending on the product, special compounds within the cell (e.g., starch), overall structure and shape of separate cells, and the structure and shape of tissues like the presence of strong vascular tissue may also contribute to texture (Van Dijk and Tijskens, 2000). The basic structure of the primary cell wall consists of a cellulose–hemicellulose network, which is chemically inert with pectin polymers interwoven with this network. The network of these three polysaccharides forms the basis for the cohesive force inside the cell. Pectin, as the main constituent of the middle lamella, cements cell walls and gives firmness and elasticity to tissues (Fuchigiami, 1987).

According to Van Dijk and Tijskens (2000), the three different polysaccharides of plant cell wall respond differently to postharvest storage and processing resulting in two components of the firmness of plant material. The firmness generated by the cellulose–hemicellulose domain of plant cell wall is not significantly affected by processing or storage while the pectin component is affected by both enzymatic and non-enzymatic reactions. Non-enzymatic depolymerization of pectin becomes significant during thermal processing at elevated temperatures ($>80^{\circ}$C, pH >4.5; Sila et al., 2009). Three enzymes are generally believed to be involved in evolution of the textural properties of plant materials, namely pectin methylesterase (PME), PG, and POD. The synergistic activity of PME and PG results in pectin modification and subsequent change in texture. The consistency and cloud stability of fruit and vegetable juices are also affected by the activity of PME and PG. POD catalyzes the oxidation of cinnamic acids and tyrosin containing cell wall proteins, which are involved in the oxidative cross-linking of cell wall polysaccharides (Van DijK and Tijskens, 2000; Van Buggenhout et al., 2009). PME and PG are discussed in this section while POD is discussed in section "Color Related Enzymes" together with enzymes that affect the color of horticultural products.

Pectin Methylesterase (PME)

Pectin methylesterase (EC 3.1.1.11) catalyzes the de-esterification of pectin to acidic pectin with a lower degree of esterification and methanol. PME is found in all higher plants and is also produced by phytopathogenic fungi and bacteria. The physiological role of PME is well established. PME is involved in fruit ripening as well as cell wall extension during cell growth (Giovane et al., 2004). PMEs from different sources exist in several isoforms, which may be distinguished from one another by their molecular weight, isoelectric point, biochemical activity, and/or stability (Plaza et al., 2007). The activity of PME destabilizes pectinaceous materials in fruit juices and concentrates (Rombouts et al., 1982; Baker and Cameron, 1999) and modifies the texture of fruit and vegetable products (Javeri and Wicker, 1991; Giovane et al., 2004) as the de-esterified pectin can be easily depolymerized by PG (Pressey and Avants, 1982). The de-esterified pectin also precipitates or gels as calcium pectinate or pectate leading to cloud loss in fruit juices and gelation of juice concentrates.

The activity of PME affects pectin and the firmness of plant tissue in three different ways. Plant PME demethoxylates pectin blockwise, which increases the probability that two adjacent polygalacturonic polymer chains form an "egg box" structure

in the presence of divalent cations such as calcium leading to an apparent increase in firmness. The second effect is that demethoxylated pectin resists β-eliminative degradation. These two effects are the basis for the application of low temperature (50–80°C) blanching with or without calcium infiltration pretreatments to reduce tissue softening during subsequent thermal processing. The treatment activates PME resulting in increased pectin demethoxylation and pectin cross-linking (Fuchigiami, 1987; Ng and Walderon, 1997; Stolle-Smits et al., 2000; Van Dijk and Tijskens, 2000). On the other hand, demethoxylated pectin is the preferred substrate for the action of PG. PG attacks the glycosidic linkages between adjacent demethoxylated galacturonic acid units of pectin resulting in pectin degradation and consequent decrease in firmness.

Fruit and vegetable juices are biphasic colloidal systems consisting of a liquid phase, termed as "serum," and a solid phase, termed as "cloud." The cloud is stabilized by the soluble pectin in the juice (Balestrieri et al., 1990). Juice cloud is an important quality attribute in products such as orange juice contributing to flavor, aroma, and color. Consumers usually associate cloud loss in products such as orange juice with spoilage and quality loss (Baker and Cameron, 1999). Thus, maintenance of juice cloud is important both from the perspective of eye appeal as well as flavor compounds associated with the cloud matrix and overall quality of the product. Juice cloud loss is caused by the activity of PME. PME catalyzes the hydrolysis of methyl ester bonds along the pectin chain to produce free carboxyl groups, which bind with divalent cations such calcium in the juice forming cross-links between adjacent pectin molecules. The cross-linked pectins form aggregates and settle resulting in juice cloud loss and phase separation (Rombouts et al., 1982; Baker and Cameron, 1999). Thermal inactivation of PME is the most common method of juice cloud stabilization. Commercial fruit products containing endogenous PME (e.g., tomato and citrus products) are commonly heat treated at temperatures between 80°C and 90°C to inactivate PME and prolong shelf life.

Polygalacturonase (PG)

Polygalacturonase (EC 3.2.1.15) catalyzes the cleavage of the α-(1-4) glycosidic bonds between two galacturonic acid residues in pectin resulting in pectin depolymerization. As mentioned above, the preferred substrate for the action of PG is demethoxylated pectin produced by the action of PME. The synergistic action of PME and PG results in the modification of pectin leading to degradation of textural quality of plant materials. A similar phenomenon is responsible for the softening of plant tissues during ripening and senescence (Balestrieri et al., 1990). PG is a cell wall-bound enzyme, which is present in many fruits and vegetables. It is also produced by plant fungal and bacterial pathogens and plays a major role in plant pathogenesis. PG exists in many isoforms with variable substrate preference, specific activity, optimal pH, and stability. Fungal PG together with fungal PME is an important component of commercial pectinolytic preparations that are used for the industrial treatment of fruit and vegetable pulps in the production of nectars and juices (Sorensen et al., 2004).

The activity of PG has been studied in many fruits and vegetables including peaches (Byun et al., 2003; Li et al., 2005; Kan et al., 2006), apples (Li et al., 2005; Goulao et al., 2007), prickly pear (Liu et al., 2004; Hernandez-Perez et al., 2005; Liu et al., 2005), papaya (Jiang et al., 2003; Manrique and Lajolo, 2004; Zainon et al., 2004), carrot (Hyeon-Gyu-Lee et al., 2001; Anthon and Barrett, 2002; Costa-Raposo-Pires and Finardi-Filho, 2005), and potato (Puri et al., 1981; Anthon and Barrett, 2002). Most of these studies are focused on the role of PG in ripening, postharvest senescence, and pathogenesis. The majority of studies with respect to the effect of processing in general and emerging technologies in particular are on tomato PG, probably due to its commercial significance and the substantial impact of PG on the rheological characteristics of tomato-based products.

The consistency of tomato products is highly dependent on pectic substances, which form a matrix in which other particles are suspended. Following tomato crushing during processing, degradation of pectin by the synergistic action of PME and PG ensues, resulting in large decrease in viscosity over a short period of time. Therefore, in conventional tomato processing, the enzymes are inactivated by thermal treatment. Two types of processes are commonly employed: hot break and cold break. In hot break process, the pulp is rapidly heated to 95–102°C immediately after crushing leading to complete inactivation of the enzymes and a high-consistency product. However, the severe heat treatment causes degradation in the color, flavor, and nutritional quality of the juice. In cold break, the pulp is heated to 60–71°C resulting in partial inactivation of the enzymes leading to low-consistency product and syneresis during storage since both enzymes are relatively heat resistant. On the other hand, quality degradation due to thermal treatment is minimized. In addition, the low viscosity of the juice makes pumping and evaporative concentration easier and reduces fouling of heat exchangers (Hayes et al., 1998; Vercet et al., 2002).

PG in tomato juice exists as a mixture of two isoenzymes: PG1 and PG2. PG2 is heat labile and is totally inactivated after five-minute treatment at 65°C. PG1 is a dimer formed from a structural association of PG2 with a heat stable glycoprotein-β subunit, which confers heat stability on it. The existence of these two isoforms in vivo or whether PG1 is an artifact of extraction is still a matter of debate (Fachin et al., 2004). Nevertheless, recent study showed that only small proportion of PG1 was found in tomato pieces as compared to tomato juice, which seems to support the hypothesis that the occurrence of PG1 is at least partially an artifact of fruit handling and processing (Peeters et al., 2003).

Color-Related Enzymes

Color is one of the main physical attributes of food products, which plays perhaps a more important role than any other

quality parameter in consumer decision of initial purchase. In addition to determining visual appeal, it can inform us about several other properties such as degree of ripeness, product alteration, etc. The main groups of pigments that are responsible for the characteristic colors of fruits and vegetables are carotenes and carotenoids, anthocyanins, chlorophylls, and phenolic compounds (Dorantes-Alvarez and Chiralt, 2000). In addition to imparting color to horticultural products, carotenoids, anthocyanins, and other phenolic compounds are potent antioxidants, which are thought to be beneficial to health. The main enzymes that are involved in biochemical degradation of plant pigments are PPO, chlorophylase, POD, LOX, and β-glucosidase (anthocyanin-β-glucosidase). Another enzyme of interest in color degradation is allinase, which is involved in the pink and green color discoloration of onion and garlic, respectively (Bai et al., 2005).

The main enzymes that are believed to be involved in the degradation of polyphenols in horticultural products and subsequent browning discoloration are PPO, POD, and β-glucosidase (Zabetakis et al., 2000). The relative effect of each enzyme depends on the product. Prevention of enzymatic browning during processing and storage of fruits and vegetables is one of the major concerns of the food industry as browning provokes a negative reaction from consumers. The exceptions include tea processing in which the activity of oxidative enzymes is welcome for enhancing a brown-black color by increasing the formation of theaflavin and thearubigin compounds and sometimes the processing of dried fruits such as dates and raisins (Tomas-Barberan and Espin, 2001).

Chlorophyll degradation during processing and postharvest storage causes a change in color from brilliant green to olive brown in processed foods and to yellow, brown, or colorless in senescent tissue (Dorantes-Alvarez and Chiralt, 2000). To date, the biochemical pathway for the degradation of chlorophyll and loss of green color has not been well characterized. Up to 20 enzymes including chlorophyllase are thought to be involved in the series of reactions that transforms chlorophyll into a number of colorless catabolites (Matile and Hortensteiner, 1999; Harpaz-Saad et al., 2007). Nevertheless, the pathway for the degradation of chlorophyll in processed products is different since chemical hydrolysis may occur at high temperature and/or low pH conditions causing chlorophyll degradation with or without the action of enzymes. Acidic substitution at low pH and heat can cause the removal of the central magnesium atom from chlorophyll leading to the formation of pheophytin followed by enzymatic (by the action of chlorophyllase) or chemical cleavage of the phytol chain to form pheophorbide (Heaton et al., 1996). The degradation of chlorophyll by POD in the presence of hydrogen peroxide and a phenolic compound and LOX in the presence of linolenic acid has also been observed in vitro (Matile and Hortensteiner, 1999). A strong correlation between yellowing in broccoli florets and increased POD and chlorophyll oxidase activity, which presumably showed the involvement of these enzymes in vivo, has been reported (Funamoto et al., 2002; Funamoto et al., 2003). In general, the degradation pathway of chlorophyll depends on the type of products with intrinsic and extrinsic factors playing a role as well as the activities of chlorophyll-degrading enzymes. Further discussion of chlorophyll-degrading enzymes will be limited to chlorophyllase.

Carotenoids are generally stable in their natural environment. Enzymes such as LOX cause the co-oxidation of carotenoids in the presence of free fatty acids through a free radical mediated mechanism, which significantly affects the color intensity of foodstuffs (Dorantes-Alvarez and Chiralt, 2000). PPO is also thought to be involved in the co-oxidation of carotenoids in the presence of polyphenols through a similar mechanism. These two reactions lead to the formation of different aroma compounds including ionones, which are found in many fruit flavors including blackberry, peach, and apricots (Aguedo et al., 2004). Thus, despite their bleaching effect, they can be beneficially used for the biosynthesis of aroma compounds, for instance from carotenoids in processing waste. PPO, POD, β-glucosidase, and chlorophyllase are discussed in detail in this section. LOX is discussed with other flavor-related enzymes in section "Enzymes Related to Off-Flavor Development."

Polyphenol Oxidase (PPO)

Polyphenol oxidase (EC 1.14.18.1) is a copper-containing enzyme, which acts on phenols in the presence of oxygen. In higher plants, it is a plastid enzyme mainly located on the thylakoid membrane of chlorophlasts as a membrane-bound protein (Carbonaro and Mattera, 2001). PPO is known by different names including tyrosinase, cresolase, catecholase, diphenolase, and phenolase. PPO catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to o-diphenols (monophenolase, cresolase, or hydroxylase activity) and the oxidation of o-diphenols to o-quinones (diphenolase, catecholase, or oxidase activity; Tomas-Barberan and Espin, 2001). The o-quinones, which are yellow in color, are highly unstable and either react with high molecular weight polymers or form macromolecular complex with amino acids and proteins (Ramaswamy and Riahi, 2003). The non-enzymatic polymerization of these intermediate compounds and condensation of o-quinones give rise to heterogeneous black, brown, or red pigments commonly called melanins (Tomas-Barberan and Espin, 2001; Ramaswamy and Riahi, 2003).

Anthocyanins are not good substrates of PPO due to their structure. The sugar moiety of anthocayanins is thought to be a steric hindrance against PPO attack. The removal of the sugar moiety by the action of β-glucosidase (specifically anthocyanase) results in the formation of anthocyanidins that can be oxidized by PPO (Zhang et al., 2005). Anthocyanins also react with the highly reactive PPO oxidation products, o-quinones, to produce brown degradation products (Zhang et al., 2005). The activity of PPO also has an impact on the flavor and aroma of horticultural products, since phenolic compounds play a role in bitter, sweet, pungent, or astringent taste of products such as apple, grape, olives, paprika, ginger, and turmeric (Tomas-Barberan and Espin, 2001). In addition to its negative effect on

phenolic antioxidants, PPO is also believed to be involved in the oxidative degradation of ascorbic acid (Talcott et al., 2003).

In principle, PPO-catalyzed browning can be prevented by inhibition or inactivation of the enzyme, transformation or removal of one of the substrates (O2 or phenols), or transformation of intermediate products to prevent melanin formation (Dorantes-Alvarez and Chiralt, 2000; Ramaswamy and Riahi, 2003). Several methods have been used to control or inhibit PPO activity in fruit and vegetable products such as thermal processing, refrigeration, lowering of the pH, and enzyme inhibitors. The method used depends on the type of product. In minimally processed fruits and vegetable products, modified atmosphere packaging together with refrigerated storage is used with or without the addition of antibrowning agents. Thermal shock treatments (40–50°C, 30–60 seconds) are also being investigated for the prevention of browning in minimally processed products to prevent chilling injury. Such treatments are investigated as a means of directing cellular metabolism away from activating the enzymes in phenolic metabolism to the synthesis of heat shock proteins (Tomas-Barberan and Espin, 2001). High temperature short time (HTST) blanching processes may also be used in minimally processed products (Dorantes-Alvarez and Chiralt, 2000). In processed horticultural products, such as juices, canned fruits, vegetables, etc., the process is designed to ensure microbial stability as well as inactivation of oxidative enzymes, and a temperature above 80°C is needed to ensure PPO inactivation (Dorantes-Alvarez and Chiralt, 2000; Tomas-Barberan and Espin, 2001).

Several chemical additives are also permitted for use as antibrowning agents. These additives can be grouped into three categories, i.e., reducing agents, chelating agents, and inorganic salts. Reducing agents such as ascorbic acid and thiol compounds act by reducing quinones into phenols. Chelating agents such as citric acid act by forming a complex with copper through an unshared electron in their structure. Inorganic salts such as sodium and calcium chloride inhibit PPO directly (Dorantes-Alvarez and Chiralt, 2000; Tortoe et al., 2007). The most commonly used reducing agents that have been used traditionally for effective prevention of enzymatic browning are sulfites. Nevertheless, due to the harmful effects of these compounds on asthmatics, their use is partly restricted or banned in some countries (Ahvenainen, 2000; Dorantes-Alvarez and Chiralt, 2000). Some "natural" alternatives to the prevention of browning are also being explored. Pineapple juice has been reported to be effective in preventing browning in apples (Lozano-de-Gonzalez et al., 1993; Perera et al., 2010). Proteases such as ficin from figs, papain from papaya, and bromelain from pineapple have also been found to be useful in preventing browning in apples and potatoes (Taoukis et al., 1989; Labuza et al., 1992).

Peroxidase (POD)

Peroxidase (EC 1.11.1.7) is found in almost all living organisms, its principal physiological function being to control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals that are harmful to all living organisms (Van Dijk and Tijskens, 2000). POD catalyzes single-electron oxidation of a wide variety of compounds in the presence of hydrogen peroxide (Tomas-Barberan and Espin, 2001). Plant POD consists of a complex spectrum of isoenzymes existing both in soluble and bound forms (Van Dijk and Tijskens, 2000). POD is believed to be involved in color and flavor degradation of horticultural products. POD catalyzes the oxidation of phenolic compounds in the presence of hydrogen peroxide leading to the formation of brown degradation products. However, whether this reaction takes place in plants is unclear since the low internal concentration in plants of hydrogen peroxide, which is essential in POD-catalyzed reactions, limits its activity.

The possible role of PPO as a promoter of POD activity is suggested since hydrogen peroxide is generated during the PPO-catalyzed oxidation of phenolic compounds (Tomas-Barberan and Espin, 2001). There are also studies that showed a high correlation between anthocyanin degradation and browning discoloration during postharvest storage with increased POD activity (Underhill and Critchley, 1995; Zhang et al., 2003; Zhang et al., 2005). Therefore, although the main agent responsible for enzymatic browning in fruits and vegetables is PPO, a possible synergistic effect between PPO and POD cannot be excluded (Tomas-Barberan and Espin, 2001). Increased POD activity has also been correlated with chlorophyll degradation in broccoli (Funamoto et al., 2002; Funamoto et al., 2003).

On the positive side, POD catalyzes the formation of phenolic cross-linkings between neighboring cell wall polymers in adjacent cells improving the textural quality of some vegetables during thermal processing (Van Dijk and Tijskens, 2000). In general, due to the degradation of pectin and the resulting cell separation, significant tissue softening occurs during thermal processing of parenchyma-rich plant tissues. However, there are some exceptions. No significant softening occurs during cooking of some vegetables including Chinese water chestnut and to a lesser extent sugar beet and beet root. This is mainly attributed to the activity of cell wall-bound POD, which catalyzes the oxidation of phenolic compounds such as ferulic acid in the cell wall in the presence of peroxides. This results in the formation of ferulic acid cross-links between cell wall polysaccharides enhancing cell to cell adhesion (Waldron et al., 1997; Ng et al., 1998; Waldron et al., 2003; Van Buggenhout et al., 2009).

POD has a relatively high thermal stability. So, it is normally used as an indicator enzyme for the sufficiency of blanching pre-treatments. It is assumed that if POD is inactivated, all the other quality-degrading enzymes are inactivated (Whitaker, 1991; Van Dijk and Tijskens, 2000). This, in many cases, implies thermal treatment in excess of what is required to inactivate the main enzyme(s) that causes quality degradation in a given product (Whitaker, 1991; Barrett et al., 2000). In addition, the thermostability of PODs from different sources varies. In fact, PODs from some plants are less thermostable than other enzymes. For instance, Morales-Blancas et al. (2002) observed that in broccoli and asparagus, LOX is more thermostable than POD. Ten-minute treatment of shredded broccoli

at 95°C resulted in complete inactivation of POD while 29.1% LOX activity remained. In the case of asparagus tip, 10-minute treatment at 95°C resulted in 85.4% inactivation of POD while 40.1% residual LOX activity was observed after such treatment (Morales-Blancas et al., 2002). The same was observed in the case of tomato where POD was found to be less thermostable than PME and PG. The *D* value at 70°C of POD in tomato juice was calculated to be 1.5 minutes compared to four minutes and 10.4 minutes for PG2 and PME (Anthon and Barrett, 2002). Therefore, complete inactivation of POD may not ensure complete enzyme inactivation and product stability in all cases.

β-Glucosidase

β-glucosidases (β-glucosidase glucohydrolase, EC 3.2.1.21) catalyze the hydrolysis of aryl and alkyl β-D-glucosides releasing β-D-glucose. These enzymes have a broad specificity for β-D-glucosides and are involved in the activation of plant defense chemicals (phytoanticipins) and conjugates of plant growth regulators by hydrolysing β-glucosidic bonds (Morant et al., 2008). They also play a significant role in the release of many volatile compounds from their glycosidic precursors in fruits and vegetables (Orruno et al., 2001). These enzymes are also increasingly finding food-processing applications. Examples include enhancing the flavor of fruit juices and fermented beverages (beer, wine, etc.; Shoseyov et al., 1989), debittering of some fruit juices by the conversion of bitter flavonone glycosides to non-bitter aglycones (Perera and Baldwin, 2001), and improving the bioavailability of isoflavones in soy products by hydrolyzing the isoflavone glycosides into the bioactive aglycones.

The enzyme anthocyanase (anthocyanin-β-glucosidase) is a specific β-glucosidase, which catalyzes the hydrolysis of sugar moieties from anthocyanins yielding highly unstable anthocyanidins (Zhang et al., 2005). Anthocyanidins are further oxidized by PPO and POD or react with o-quinones to form melanins (Zhang et al., 2003; Zhang et al., 2005). Thus, the activity of anthocyanase needs to be controlled during postharvest storage and processing of anthocyanin-rich products such as berry fruits. No information is available in the open literature on the control of the activity of anthocyanase or the effect of thermal treatment on anthocyanase from higher plants.

Chlorophyllase

Chlorophyllase is a glycoprotein, which is located in the plastid envelope of green plants (Matile and Hortensteiner, 1999). Chlorophyllase (Chlase, E.C. 3.1.1.14) catalyzes the hydrolysis of chlorophyll into chlorophyllide and phytol, which is the first step in the biochemical degradation of chlorophyll. Chlorophyllase has been found to be the rate-limiting enzyme in chlorophyll catabolism during ripening, senescence, seasonal changes, as well as natural turnover (Harpaz-Saad et al., 2007). A clear correlation has been reported between increased chlorophyllase activity and chlorophyll degradation in prickly pear cactus stems (Guevara et al., 2003). The activity of chlorophyllase is also implicated in the degradation of chlorophyll in coleslaw cabbage (Heaton et al., 1996) whereas chlorophyll degradation in broccoli has been partly attributed to the activities of chlorophyll degrading POD and chlorophyll oxidase as well as chlorophyllase (Funamoto et al., 2002; Funamoto et al., 2003). There is also a growing interest in the application of chlorophyllase as a biocatalyst for removing green pigments from edible oils as an alternative to conventional bleaching techniques (Bitar et al., 2004).

Thermal processing (blanching and canning) is the principal way of controlling enzyme activity. However, as mentioned above, chlorophyll loses its phytol chain during heating and loss of magnesium occurs in acidic media resulting in a dirty brown discoloration of the product. Thus, blanching is done in slightly alkaline media or by adding magnesium salts in a non-acid medium. On the other hand, in products containing both chlorophyll and anthocyanin, this approach may have undesirable consequence as anthocyanins turn brown in alkaline media (Dorantes-Alvarez and Chiralt, 2000). With respect to minimally processed fruits and vegetables, modified atmospheric packaging with high concentration of CO_2 (Guevara et al., 2003), use of immersion solutions containing chemicals such as citric acid to shift the pH from the optimal range for chlorophyllase activity (Ihl et al., 2003), ethylene diamine tetracetic acid (EDTA) to stabilize magnesium through their chelating action (Sanchez et al., 1991), and prolonged thermal treatment at mild temperature (eg. two hours at 50°C) (Funamoto et al., 2002; Funamoto et al., 2003) have been found useful for controlling the activity of chlorophyllase and delaying chlorophyll degradation.

Alliinase

Alliinase (cysteine sulphoxide lyase; C-S lyase, EC 4.4.1.4) hydrolyzes the non-protein amino acid, *S*-allyl-*L*-cysteine sulfoxide (alliin) into pyruvate, ammonia, and diallyl thiosulphinate (allicin), the most important compound in garlic, which is responsible for its characteristic pungent flavor and its therapeutic and antimicrobial effects. Alliinase is also the enzyme that catalyzes the hydolysis of *S*-(1-propenyl)-*L*-cysteine sulfoxide (isoalliin) into di(1-propenyl) thiosulfinate, which gives onion its flavor. However, this same enzyme is believed to be involved in the green discoloration of processed garlic products as well as pink discoloration in onion. In both cases, the discoloration process has two steps. In the first step, alliinase acts on alk(en)yl-*L*-cysteine sulfoxides to produce soluble organosulfur compounds called "color developers." In the second phase, these compounds react non-enzymatically with amino acids and carbonyl compounds to form pigments (Bai et al., 2005; Lee et al., 2007). This hypothesis appears to be supported by a recent study in a model system that consisted of thiosulfinates from garlic, leek, and onion, glycin, and allinase where pink, pink-magneta, and dark blue pigment formation was observed at pH 5.5 depending on the source of the thiosulfinates (Kubec et al., 2004).

In the processing of products such as pickled garlic, blanching treatment is used to inactivate allinase so as to prevent undesirable effects such as pungent flavor and green color development during product storage. Blanching treatment of 11 minutes at 80°C is sufficient to prevent the formation of pungent flavor as well as green discoloration due to alliinase activity in pickled garlic (Rejano et al., 2004). However, the activity of alliinase is required for the formation of beneficial compounds such as allicin in garlic, which are responsible for both the flavor and therapeutic properties of garlic. In this case, the challenge is to keep the enzyme active while inhibiting the non-enzymatic step of the green pigment formation reaction. This appears to be possible by maintaining the pH close to neutral, since low pH (2.0–3.0) favors the non-enzymatic reactions (Bai et al., 2005).

Enzymes Related to Off-Flavor Development

Flavor is another important quality parameter that determines the suitability of a food product for consumption. It is usually difficult to characterize the critical combination of compounds that contribute to the characteristic flavor and aroma of food products and identify the enzymes responsible for the biosynthesis of flavor compounds and off-flavor development (Whitaker, 1996). Enzymes can cause off-flavor development in foods especially during storage. Vegetables such as green beans, green peas, corn, broccoli, and cauliflower develop off-flavor during frozen storage if they are not properly blanched. According to Whitaker and co-workers, LOX is the main enzyme responsible for off-flavor development in green beans, green peas, and corn while cystine lyase is responsible for off-flavor development in broccoli and cauliflower (Whitaker, 1996; Ramirez and Whitaker, 1998b; Barrett et al., 2000). These authors suggest that better quality products can be obtained if these enzymes are used as indicators of the adequacy of blanching treatment instead of POD. However, effective blanching may not be a guarantee against off-flavor development. A recent study showed that thermally inactivated POD is responsible for lipid oxidation and off-flavor development in blanched vegetables such as cauliflower. This was proposed to be due to calcium abstraction from the enzyme that increases the exposure of lipids to the haem group in the denatured enzyme (Adams et al., 2003).

Lipoxygenase (LOX)

Lipoxygenase (E.C. 1.3.11.12) catalyzes the oxidation of polyunsaturated fatty acids and esters into the corresponding hydroperoxides (Ludikhuyze et al., 1998c; Rodrigo et al., 2006b). There are many isoenzymes of LOXs in plant tissue that differ in pH optimum, isoelectric point, and other properties. The physiological role of LOX is not sufficiently understood although metabolites of unsaturated fatty acids have been implicated in growth and development, plant senescence, and response to disease and wounding. LOX also plays a major role in the formation of volatile flavor compounds in fruits and vegetables responsible for the "fresh" and "green" sensorial note (Rodrigo et al., 2006b, 2007). LOX catalyzes the oxidation of polyunsaturated fatty acids into hydroperoxides, while HPL catalyzes the cleavage of 13-hydroperoxy lipids into six carbon aldehydes. A selective lyase cleavage of hydroperoxides leads to the formation of hexanal and hexenel from the 13-hydroperoxides of linoleic and linolenic acids, respectively. The aldehydes formed are considered to be the major volatile compounds that contribute to the "fresh" flavor of fruits and vegetables. The C6 aldehydes are also converted into their corresponding alcohols through the action of alcohol dehydrogenase. This series of reactions is often called the "LOX pathway." The aldehydes and alcohols formed in the LOX pathway are also important in the aroma of vegetable-derived products such as olive oil.

On the other hand, the LOX-catalyzed oxidation of unsaturated fatty acids, which involves the formation of free radical intermediates, has at least three detrimental effects on food quality (Whitaker, 1996): (1) loss of nutritional quality due to the destruction of the essential fatty acids lineoleic, linolenic, and arachidonic acids as a direct consequence of the reaction and indirect degradation of vitamins and proteins by hydroperoxides and the free radical intermediates; (2) development of off-flavor often characterized as hay-like, which is due to further degradation of the hydroperoxides into volatile compounds such as aldehydes, ketones, and alcohols; and (3) loss of color due to the degradation of chlorophyll and carotenes by the hydroperoxides and the free radicals (Whitaker, 1996; Ludikhuyze et al., 1998c). The conventional thermal treatment, blanching, is commonly used to inactivate this enzyme as well as other quality-degrading enzymes in vegetables including beans and peas prior to freezing.

Hydroperoxide lyase (HPL)

As mentioned earlier, HPL is one of the key enzymes in the "LOX pathway" responsible for the synthesis of volatile compounds that contribute to the "green" and "fresh" sensory notes of ripe fruits and vegetables. In this respect, HPL from various sources including tomato (Rodrigo et al., 2007), olive (Luaces et al., 2007), bell pepper (Santiago-Gómez et al., 2007), and strawberries (Leone et al., 2006) have been characterized. Though the flavor compounds produced through the LOX pathway are essential, their concentration above the normal level results in off-flavor development. Thus, control of the activities of the enzymes involved including HPL is essential (Leone et al., 2006). On the other hand, HPL and LOX can be beneficially used in the synthesis of "green" flavor compounds for re-introducing into processed products as these flavor compounds are often lost during processing (Nemeth et al., 2004; Santiago-Gómez et al., 2007). There are not many studies on the thermal inactivation kinetics of HPL. The thermal inactivation of tomato HPL was described by a biphasic kinetic model indicating the presence of isozymes of different thermostability (Rodrigo et al., 2007). In the case of olive, two HPL isozymes have been identified. However, the inactivation kinetics was described by a

first-order model, which indicates that the two isozymes have similar thermostability (Luaces et al., 2007).

Cystine Lyase

Cystine lyase (cystathionine *L*-homocysteine-lyase; EC 4.4.1.8) is an enzyme that cleaves *L*-cystine producing thiocysteine (cysteine persulfide), pyruvate, and ammonia through β-elimination reaction (Ramirez and Whitaker, 1998a; Jones et al., 2003). This enzyme and alliinase belong to the group of enzymes collectively called C-S lyases that cleave alkyl-cysteines and alkyl-cysteine sulfoxides, which are the major constituents of the free amino acid pool of many vegetables of the family cruciferae, luguminosae, and liliacea. The reactions catalyzed by C-S lyases together with other secondary reactions are responsible for the desirable and undesirable flavor and aromas in these vegetables (Chin and Lindsay, 1994; Ramirez and Whitaker, 1998a).

Cystine lyase has been identified in several members of the Brassica genus such as cabbage, cauliflower, kale, mustard, turnip, and rutabaga (Ramirez and Whitaker, 1999). This enzyme has been determined to be the principal cause of off-flavor development in broccoli and cauliflower (Lim et al., 1989; Whitaker, 1996) and the complete inactivation of this enzyme has been suggested as a more suitable indicator of sufficient blanching treatment of these vegetables since it is more thermolabile than POD (Ramirez and Whitaker, 1998b).

The activities of cystine and C-S lyases were also reported to be responsible for the formation of undesirable sulphur compounds such as methanethiol, dimethyl disulfide, and dimethyl trisulfide in disrupted tissues of Brassica vegetables such as cabbage and broccoli (Forney et al., 1991; Chin and Lindsay, 1993; Obenland and Aung, 1996). This is a problem especially in the application of modified atmosphere packaging for the preservation of such vegetables as the unpleasant sulphurous odor limits consumer acceptance (Forney et al., 1991; Ramirez and Whitaker, 1998a). The formation of dimethyl trisulfide occurred only under anaerobic conditions (Chin and Lindsay, 1994). Excessively low O_2 concentration also led to increased production of methanethiol and the formation of an offensive flavor (Obenland and Aung, 1994). Different chemical treatments are used to limit the formation of these undesirable volatiles in broccoli stored under modified atmospheric condition such as dipping in tertiary butylhydroquinone, ascorbic acid, phosphoric acid and sodium hydroxide, caraway seed extract, and hydroxylamine (a cystine lyase inhibitor; Obenland and Aung, 1996; Ramirez and Whitaker, 1998a).

Enzymes Responsible for Changes in Nutritional Quality

Relatively little information is available on the enzymes that affect the nutritional quality of horticultural products except on those that are involved in other quality degradations. As discussed in section "LOX," the activity of LOX causes decrease in the amounts of essential fatty acids such as linoleic, linolenic, and archidonic acids. The free radicals formed during LOX-catalyzed oxidation of polyunsaturated fatty acids cause oxidation of carotenoids (vitamin A precursors as well as potent antioxidants), tocopherols (vitamin E), ascorbic acid, folate and cysteine, tyrosine, tryptophan, and histidine residues of proteins. The activity of PPO, POD, and anthocyanase cause degradation of polyphenols including anthocyanins that are important phytochemicals. Enzymatic browning caused by these enzymes also results in the decrease of the available lysine content of proteins. Ascorbic acid oxidase causes degradation of ascorbic acid in vegetables such as squash. Thiaminase causes the degradation of thiamine that is an essential co-factor in amino acid metabolism. Riboflavin hydrolase causes degradation of riboflavin. However, it is only found in some microorganisms (Whitaker, 1996). On the other hand, myrosinase enhances the nutritional quality of Brassica vegetables as it converts glucosinolates into the biologically active forms such as thiocynates and isothiocynates.

Ascorbic acid oxidase catalyzes the transformation of ascorbic acid into dehydroascorbic acid, which has the same physiological efficiency as ascorbic acid. Further degradation of dehydroascorbic acid into diketogluconic acid, oxalic acid, and other nutritionally inactive compounds may take place. However, this was reported to occur only at high temperature. In addition, the enzyme is believed to be inactivated once it is ingested in the gastro-intestinal tract (Kiribuchi and Kawashima, 1987). Thiaminase catalyzes the replacement of the thiazole moiety of thiamine with a variety of nucleophiles (Campobasso et al., 1998). The activity of this enzyme has been observed in marine organisms, silk worm, bacteria, and plants (Campobasso et al., 1998; Nishimune et al., 2000). Although, ingestion of a significant quantity of thiaminase may lead to a serious thiamine deficiency even when there is sufficient amount of thiamine in the diet (Vimokesant et al., 1982; Nishimune et al., 2000), the occurrence of the enzyme is limited to certain plants that are not normally consumed by humans except a few like *Marsilea drummondii* that is consumed cooked by Australian Aborigines and fishes that are commonly eaten after cooking and the enzyme is inactivated. Therefore, further discussion will be limited to myrosinase.

Myrosinase

Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) is an enzyme that is found in all glucosinolate-containing plants including cabbage, brussels sprout, radish, turnip, water cress, and mustard, which are important in human diet either as vegetables or condiments. It is also found in insects, fungi, and bacteria. Several isozymes of myrosinases have been observed. Myrosinase catalyzes the hydrolysis of glucosinolates, into D-glucose and an aglucone, which spontaneously convert into isothiocyanates or indoles depending on the side chain—the biologically active forms of glucosinolates, which are believed to be anticarcinogenic and play a role in the prevention of

cardiovascular diseases. Isothiocyanates are also responsible for the typical odor and taste of cruciferous vegetables. Depending on the pH, presence of ferrous ions, double bond in the side chains, and proteins such as epithiospecifier protein (ESP), further conversion of isothiocyanates and indoles into epthionitriles, nitriles, thiocynates, and other compounds occur. The nitriles formed do not have the beneficial health effect of isothiocyanates. The main factor that determines the isothiocynates–nitrile ratio is ESP. A higher proportion of isothiocyantes is obtained if processing of cruciferous vegetables is carried out at a condition where ESP is inactivated while the activity of myrosinase is maintained (Eylen et al., 2007).

Conventional thermal processing such as blanching inactivates myrosinase before the glucosinolates are transformed into the biologically active forms. Myrosinase is a relatively thermolabile enzyme. For instance, broccoli myrosinase is inactivated in the temperature range between 40°C and 60°C. In the human gut, glucosinolates can be hydrolyzed by microbial myrosinase (Barret, 2007; Van Eylen et al., 2007). However, this appears to be insufficient since several studies in humans have found that inactivation of myrosinase in cruciferous vegetables prior to ingestion substantially decreases the bioavailability of isothiocyanates (Shapiro et al., 2001).

HIGH-PRESSURE PROCESSING

Introduction

High-pressure processing of foods is gaining popularity in the food industry because of its ability to inactivate vegetative cells of microorganisms and some enzymes near room temperature, resulting in the almost complete retention of nutritional and sensory characteristics of fresh food without sacrificing shelf life (Tauscher, 1995; Knorr and Heinz, 2001). HPP has been used for decades with success in chemical, ceramic, carbon allotropy, steel/alloy, composite materials, and plastic industries. HPP of foods uses similar concepts to cold isostatic pressing of metals and ceramics, except that it demands much higher pressure, faster cycling, higher capacity, and hygienic design with effective cleaning routines (Mertens and Deplace, 1993; Zimmerman and Bergman, 1993).

The first scientific investigation on the application of high pressure in food preservation was by Hite in the last decade of the 19th century, who reported the application of high pressure for milk preservation (Hite, 1899) and the preservation of fruits and vegetables (Hite et al., 1914). However, it was not until the 1980s that a widespread interest in the technology was renewed leading to several studies on the various aspects of the process (Heinz and Buckow, 2010). The observation that high pressure inactivates microorganisms and reduces the activity of many quality-related food enzymes, while retaining other quality attributes, led to the introduction of a number of high-pressure processed foods into the market by Japanese and American food companies (Mermelstein, 1997; Hendrickx et al., 1998). The first high-pressure processed foods introduced into the market were jams, jellies, and sauces in Japan in 1990 by the Japanese company Meidi-ya (Thakur and Nelson, 1998). This was followed by fruit preparations, fruit juices, rice cakes, and raw squid in Japan; apple and orange juices in France and Portugal; guacamole and oysters in the USA; and processed meat products in the USA and Spain (Hugas et al., 2002). Since 2000, a four-fold increase in the number of commercial high-pressure installations has occurred with about 128 industrial scale high-pressure equipments in operation in 2009 (Heinz and Buckow, 2010). Consumers are increasingly looking for and are willing to pay more for safe "fresh-like" food products processed with minimal heat and chemical preservatives, which is possible with HPP. Therefore, the growth trend in the commercial application of HPP is set to continue in the foreseeable future. Currently, the cost of HPP ranges from 8 to 50 cents (US $) per kg depending on the operating parameters and the scale of operation, including depreciation and operating costs. With increasing demand and innovations in equipment design, further decrease is expected in both capital and operating costs (Balasubramaniam et al., 2008).

HPP above 200 MPa inactivates vegetative bacteria, yeast, and moulds. In practice, pressures up to 700 MPa and treatment times from a few seconds to several minutes are used to inactivate microbial cells. Bacterial spores on the other hand are highly resistant to pressure showing a remarkable tolerance to pressures above 1000 MPa near room temperature (Margosch et al., 2004; Margosch et al., 2006). Nevertheless, sterilization of low-acid foods is possible through combined high pressure (500–900 MPa) and relatively mild temperature (90–120°C) processing for about five minutes. Such HPP-assisted thermal sterlization process has been approved by the FDA for a shelf-stable high quality mashed potato product (Somerville and Balasubramaniam, 2009). HPP has the potential to deliver preservative-free, fresh-like food products that are safe and have good shelf life. Several studies have shown that high pressure does only minimally affect physicochemical quality attributes such as flavor and color. In addition, nutrients such as vitamin A and phytochemicals such as carotenoids and polyphenols are not significantly affected by HPP near room temperature (Oey et al., 2008). High retention (up to 85% or more depending on the temperature) of water soluble vitamins such as vitamins C, B1, and B6 has also been reported (Sanchez-Moreno et al., 2009).

The Effects of High Pressure on Enzymes

The application of pressure above 300 MPa can cause irreversible protein denaturation at room temperature, while lower pressure results in largely reversible changes in protein structure (Knorr et al., 2006). A lowering of the denaturation pressure both as the temperature is increased and decreased (cold denaturation) has been observed (Hendrickx et al., 1998). Enzymes are special type of proteins whose biological activity arises from

an active site brought about by the three-dimensional configuration of the molecule. Denaturation of the enzyme with the accompanying conformational changes can alter the functionality of the enzyme resulting in increase or loss of biological activity and changes in substrate specificity (Hendrickx et al., 1998). Nevertheless, loss of activity may occur without significant change in the conformation of the enzyme (Heinisch et al., 1995; Ludikhuyze et al., 1998b).

It has long been known that proteins show changes in their native structure under high pressure similar to the changes occurring at high temperatures (Bridgman, 1914). The native confirmation of proteins is stable only in a narrow range of pressure and temperature. The structure of a protein is stabilized by a sensitive balance of disulfide bonds and various non-covalent forces such as hydrogen bonds and hydrophobic, electrostatic, and van der Waals interactions. Upon heating, all these forces are weakened, particularly due to molecular fluctuations, and the protein molecule denatures or unfolds. The amino acid residues that form the active center of an enzyme are brought together only in the native structure of the molecule and unfolding results in disassembling of this domain and thus loss of enzyme activity. The mechanism of pressure-induced enzyme inactivation is different to that of heat inactivation. Contrary to thermal inactivation, pressure inactivation near room temperature is usually not accompanied by changes in covalent bonding. In aqueous solution, pressure affects mainly the tertiary and quaternary structure of proteins. Oligomeric proteins have been found to dissociate into individual subunits even at about 150–200 MPa (Balny and Masson, 1993; Knudsen et al., 2004). Imperfect packaging of atoms at the subunit interface together with the disruption of hydrophobic and/or electrostatic interactions in the subunit area might account for the volume changes involved (Penniston, 1971; Mozhaev et al., 1994). The disturbance of the tertiary structure, which is predominantly stabilized by hydrophobic and electrostatic interaction, usually takes place at pressures beyond 150–200 MPa (Cheftel, 1992; Balny and Masson, 1993).

In contrast to temperature, which destabilizes the protein molecule by transferring non-polar hydrocarbons from the hydrophobic core towards the water, pressure denaturation is initiated by water penetration into the interior of the protein molecule (Nash and Jonas, 1997). Partial unfolding of the protein molecule can be caused by a loss of contact between groups in the non-polar domains. Hence, the pressure stability of an enzyme is largely affected by its ability to compensate losses of non-covalent bonds due to relocation of water molecules (Boonyaratanakornkit et al., 2002; Smeller, 2002). High pressure may cause inactivation or activation of enzymes depending on the applied pressure and the type of enzyme. Application of pressure less than 100 MPa has been observed to activate some enzymes, especially monomeric enzymes such as chymotrypsin and PPO (Buckow et al., 2009). Higher pressure usually induces irreversible or reversible inactivation with the degree of reversibility depending on the degree of distortion of the molecule (Hendrickx et al., 1998; Pilar Cano and Ancos, 2005). However, some exceptions have been observed where significant increase in the activity of enzymes such as PPO occurred after treatment at pressure as high 700 MPa at ambient temperature conditions (Terefe et al., 2009b). High pressure-induced inactivation of enzymes also depends on intrinsic and extrinsic factors such as the type and origin of the enzyme, pH, medium composition, and temperature (Ludikhuyze et al., 1996; Weemaes et al., 1997a). The presence of a substrate may have protective effects but can also lead to a strong destabilization of a protein (Fernandez-Garcia et al., 2002). The changes of water properties with increasing pressure, temperature, and the presence or absence of solutes reflect changes in the arrangement of water molecules that might explain the baro-protective effects of solutes on proteins under denaturing conditions.

Moderate high pressure can be used to enhance beneficial enzyme reactions in plant tissue as it allows increased enzyme-substrate contact due to tissue disruption. It is also not unusual that the thermostability of enzymes is increased under specific pressures, allowing enzyme-catalyzed reactions to take place at moderately high temperature and faster rate (Ludikhuyze et al., 2003; Knorr et al., 2006). This can happen since van der Waals forces and hydrogen bond formation within the protein is promoted under pressure causing a reduction in volume of the protein (Gross and Jaenicke, 1994; Boonyaratanakornkit et al., 2002). The opposing effects of pressure and temperature on hydrophobic interactions and hydrogen bonds formation have been put forward as possible explanations for pressure-induced stabilization of enzymes and proteins against thermal denaturation (Mozhaev, 1996). Pressure may also affect enzyme–substrate interaction by changing the conformation of a macromolecular substrate causing an enhancement or inhibition of the enzyme-catalyzed reaction (Pilar Cano and Ancos, 2005). Moderate high pressure can also induce stress response reactions in plant cells such as increased synthesis of phenylalanine ammonia lyase (PAL) leading to increased synthesis of polyphenols (Dörnenburg and Knorr, 1997), which may be beneficial for some applications.

Effects of High Pressure on Quality-Related Plant Enzymes

PME. There are several studies on the effect of HPP on PMEs of different plant sources such as tomato, orange, banana, carrot, apple, citrus fruits, strawberries, plums, and capsicum. The most representative studies are summarized in Table 1. Depending on the origin and medium used for enzyme inactivation, threshold pressures for inactivation have been found to vary from 100 MPa to 800 MPa at room temperature. Among the PMEs investigated, tomato PME appears to be the most pressure resistant with no inactivation at ambient condition up to 800 MPa, while orange PME is the least pressure resistant with the inactivation of the labile fraction at room temperature at pressures as low as 100 MPa (see Table 1). Strawberry PME has also been reported to be quite pressure resistant with the inactivation of the labile form possible only at 850 MPa at 10°C. A very pressure stable isoform of PME has also been reported in

Table 1 Summary of representative studies on the effect of high pressure on PMEs from different sources

Source	Medium	Conditions investigated	Effect	Reference
Apple	Cloudy apple (golden delicious) juice	200–600 MPa, 15–65°C, 0.5–10.5 min	Activity increase up to 40°C at all conditions; some inactivation at higher temperatures	Baron et al. (2006)
	Purified in citrate-phosphate buffer (pH 4.0)	150–650 MPa, 25°C, 10 min	32% and 39% inactivation at 100 MPa and 650 MPa	
Banana	Purified in Tris-HCl buffer (pH 7.0)	10°C, 600–700 MPa	Inactivation of the labile fraction (92%) at $P^a \geq 600$ MPa at 10°C; inactivation at $P \geq 800$ MPa at room temperature; no inactivation of the stable fraction up to 900 MPa; antagonistic effects at $P \leq 300$–400 MPa, $T^b \geq 64$°C	Ly-Nguyen et al. (2002a)
		30–76°C, 0.1–900 MPa		Ly-Nguyen et al. (2003b)
Carrot	Carrot juice	100–600 MPa, 25°C, 10 min	40% inactivation at 100 MPa, 25°C, 10 min. Increased activity at $P = 500$–600 MPa with maximum 28.8% activation at 600 MPa	Kim et al. (2001)
		300–500 MPa, 50–70°C, 10 min	>80% inactivation ($P = 300$–500 MPa, $T = 50$–70°C). Maximum 97.6% inactivation at 500 MPa, 50°C	
Carrot	Partially purified in Tris-HCl buffer (pH 7.0)	100–825 MPa, 10–65°C, kinetic	Inactivation of the labile fraction (97%); inactivation pressure at 10°C: 600 MPa; antagonistic effect at $P < 300$ MPa and $T > 50$°C	Ly-Nguyen et al. (2002b, 2003a)
Carrot	Purified PME reconstituted into Carrot juice	700–800 MPa, 10°C	Inactivation	
	Carrot pieces	700–800 MPa, 10°C and 40°C	No inactivation at 10°C; inactivation at 800 MPa, 40°C	Balogh et al. (2004)
Grapefruit	Grapefruit juice	500–900 MPa, $T = 20$–50°C not controlled, temperature effect not accounted for	50% to a maximum of 85% inactivation (only the labile isoform) after 1 sec at 600–900 MPa	Goodner et al. (1998)
Grapefruit	Purified PME in Tris buffer (pH 7.0)	10–62°C, 0.1–800 MPa, kinetic	Only the labile form (80%) inactivated up to 800 MPa; inactivation at room temperature at $P \geq 700$ MPa; antagonistic effects at $P \leq 300$ MPa, $T \geq 58$°C	Guiavarc'h et al. (2005)
Guava	30% Guava juice (pH 4.7, 3° Brix)	600 MPa, ambient temperature	600 MPa, 10 min; Partial inactivation (45%)	Lin and Yen (1995)
Orange	Purified PME (from Navel Oranges) in citric acid buffer (pH 3.7) Commercial Orange peel PME in: - Deionized water (pH 4.5) - Citric acid buffer (pH 3.7) - 1 M $CaCl_2$ soln	Labile (0.1–900 MPa, 15–65°C), stable (0.1–900 MPa, 70–82°C), kinetic	Only the labile form inactivated (92–97%); faster inactivation of extracted PME than commercial PME in deionized water; 700–900 MPa at room temperature required for inactivation; antagonistic effect at $T \geq 57$ and $P \leq 300$ MPa; commercial PME more pressure sensitive at pH 3.7 (inactivation at 400 MPa at room temperature). Inactivation of the stable fraction possible at $T \geq 70$°C; antagonistic effect of pressure at all temperatures	Van den Broeck et al. (2000a)
Orange	Orange juice (pH 3.45)	500–900 MPa, 20–50°C not controlled, temperature effect not accounted for	10–93% inactivation, respectively, after 1 sec at 600–900 MPa (only the labile isoenzyme); slower inactivation at 500 MPa	Goodner et al. (1998)
Orange	Orange juice	400–600 MPa, 25–50°C, kinetic 500 MPa, 600 MPa, 800 MPa, 25–50°C	Inactivation of the labile form at 400 MPa and 25°C; accelerated inactivation with increased temperature to 50°C; 93.6% inactivation after 1 min at 800 MPa and 25°C	Nienaber and Shellhammer (2001a, 2001b)
Orange	Greek Navel Orange juice	100–800 MPa, 30–60°C, kinetic	Inactivation of the labile form at all conditions including 100 MPa and 30°C; antagonistic effect at low pressure (100–250 MPa) and higher temperatures (60°C)	Polydera et al. (2004)

Pepper (*Capsicum annum*)	Crude extract (pH 5.6)	400–800 MPa, 25°C and 60°C, 15 min	Inactivation at $P \geq 600$ MPa; 44% and 49% inactivation at 25°C and 60°C.	Castro et al. (2005, 2006b)
	Purified (pH 5.6)			
	Pepper pieces	0.1–500 MPa, 25, 40, and 60 °C, 15 min	Activity increase at 25°C and at 40°C up to 300 MPa; inactivation at 60°C at $P \geq 300$ MPa PME more stable in purified form followed by crude extract, puree, and pieces.	
	Pepper puree			
	Purified in citrate buffer (pH 5.6)	0.1–800 MPa, 10–64°C, kinetic	Inactivation of the labile fraction (80%) at $P > 600$ MPa at room temperature; antagonistic effects at $P \leq 300$ MPa, $T > 54$°C; stable fraction inactivated at $P = 800$ MPa	
Plum	Purified in Tris-HCl buffer (pH 7.5)	400–800 MPa, 25°C, kinetic	Inactivation at $P > 600$ MPa at room temperature	Nunes et al. (2006)
Strawberry	Purified in Tris-HCl buffer (pH 7.0)	850–1000 MPa, 10°C, kinetic	Inactivation of the labile fraction (90%) at $P \geq 850$ at 10°C; no inactivation of the stable fraction up to 1000 MPa	Ly-Nguyen et al. (2002c)
Tomato	Tomato dices	400 MPa, 600 MPa, 800 MPa, 25°C and 45°C	No inactivation after up to 5 min treatment at all conditions; 50% activation after 5 min at 400 MPa and 45°C	Shook et al. (2001)
Tomato	Tomato juice	0.1–800 MPa, 60–75°C	Inactivation; anatagonistic effect with temperature with minimum inactivation at $P \leq 300$–600 MPa	Crelier et al. (2001)
Tomato	Purified PME in citrate buffer (pH 4.4)	550–700 MPa, 25°C	No inactivation	Fachin et al. (2002a)
	Tomato juice	550–700 MPa, 25°C	No inactivation	
Tomato	Crude extract in extraction soln (1.2 M NaCl)	0.1–500 MPa, –26°C to 20°C, 13 min	No inactivation	Van Buggenhout et al. (2006b)
	Tomato pieces		No inactivation	

[a] P: Pressure.
[b] T: Temperature.

many fruits and vegetables, which appears to be the same as the thermostable PME isoenzyme due to similarities in their concentration and molar mass. Most of the studies on the effects of HPP on PME have been performed on the purified, thermolabile major isoforms in buffer solutions such as citrate and Tris-HCl buffer. This limits the applicability to real food system, since (1) environmental factors such as pH, sugars, salts, or other food components may significantly affect the enzyme's activity and stability (Ogawa et al., 1990; Ludikhuyze et al., 1996; Weemaes et al., 1997a; Riahi and Ramaswamy, 2004), and (2) the destabilization of juice clouds and viscosity is mainly attributed to the thermostable isoform that is active at low temperatures (4°C) and low pH (Versteeg et al., 1980; Seymour et al., 1991).

Goodner et al. (1998) reported an instantaneous inactivation of the heat labile isoform of orange and grapefruit PME at pressures higher than 600 MPa whereas the heat stable isoform was gradually inactivated with longer treatment times. Heat labile grapefruit PME was more sensitive to pressure than orange PME. Pressurization at 700 MPa and approximately 20°C resulted in an instant loss of 61% and 82% of total PME activity in orange and grapefruit juice, respectively. Baron et al. (2006) reported similar instantaneous inactivation of the thermolabile isoform of apple PME. They observed 61% and 68% inactivation of purified apple PME in citric-phosphate buffer (pH 4.0) at 100 Mpa and 650 Mpa, respectively, at 20°C regardless of the treatment time, which appears to be due to the instantaneous pressure inactivation of the pressure labile fraction as in the case of orange and grape fruit PMEs.

Pressure inactivation of tomato PME was investigated by a large number of researchers. It is reported that tomato PME in situ or in purified form (dissolved in a buffer solution) is a heat labile enzyme at atmospheric conditions, which is significantly stabilized against thermal denaturation at pressures up to 500–600 MPa (Tangwongchai and Ames, 2000; Stoforos et al., 2002; Van den Broeck et al., 2000b; Crelier et al., 2001; Shook et al., 2001; Fachin et al., 2002a). For example, at 600 Mpa, tomato PME was stable at 60°C and retained 90% of its activity after 30 minutes at 75°C and 600 MPa (Crelier et al., 2001; Fachin et al., 2002a). The enzyme was also found to be very pressure stable up to 800 MPa at temperatures lower than 60°C (Van den Broeck et al., 2000b; Crelier et al., 2001; Fachin et al., 2002a; Rodrigo et al., 2006a). These earlier studies have focused on the effect of high pressure on the main tomato PME isoenzyme. Recently, Plaza et al. (2007) identified four tomato PME isoenzymes and investigated the thermal and pressure stability of the two major isoenzymes. They observed a striking difference between the pressure stabilities of the main heat stable isofraction of tomato PME and the minor (about 15% of total PME activity) heat sensitive isofraction. The threshold pressure for inactivation of the heat labile isoenzyme in buffer solution (pH 6.0) was approximately 500 MPa at 20°C compared to approximately 850 MPa for the heat stable isoenzyme. The determined D values for heat labile PME at 600 MPa and 20°C or 40°C were 18.5 and 6.2 minutes, respectively (Plaza et al., 2007).

In general, HPP at practically feasible conditions does not result in complete inactivation of PME. However, good cloud stability in high pressure-treated juices and purees have been reported in many cases even though significant PME activity remained following processing. Orange juice pressurized at 700 MPa for one minute had no cloud loss for more than 50 days under refrigerated storage conditions, despite the 18% residual PME activity (Goodner et al., 1998). Similar results have been reported for orange juice processed at 800 MPa for one minute and at 900 MPa for 0.5 minute at room temperature; both resulted in low level of residual PME activity (3.9% and 5%, respectively), and good cloud stability at 4°C and 37°C for a period of more than two months was obtained (Nienaber and Shellhammer, 2001a). Pressure treatment of guava puree at 600 MPa and 25°C for 15 minutes reduced PME activity by approximately 25%, whereas a heat treatment at 90°C for 24 seconds inactivated approximately 95%. However, pressure-treated guava puree exhibited better color retention, pectin, cloud, and ascorbic acid content during 40 days storage at 4°C compared with heat treated samples (Yen and Lin, 1996). It seems that the shear force generated during decompression causes changes in the size and structure of the suspended solid particles, leading to better cloud stability. Lacroix et al. (2005) reported that dynamic HPP improved the cloud stability of orange juice during storage in the presence of active PME, which they attributed to the observed particle size reduction and the structural modification of pectin making it less accessible to PME following the treatment (Lacroix et al., 2005). Similar cloud stabilizations of orange juice cloud were observed following high pressure CO_2 processing (Arreola et al., 1991; Kincal et al., 2006) and ultrasonication (Tiwari et al., 2009). Mizrah and Berk (1970) observed that suspended particles below a size of 2 μm constitute the stable cloud in orange juice, and heat treatment stabilizes juice cloud not only through PME inactivation but also through particle size reduction (Mizrahi and Berk, 1970).

PG. Among the plant enzymes investigated so far, PG is one of the most pressure labile enzymes. The threshold pressure for inactivation of crude tomato PG (40 mM Na-acetate, pH 4.4) was approximately 300 MPa at 20°C (Rodrigo et al., 2006a). The application of high pressures induces conformational changes causing inactivation and/or a significant decrease of the catalytic activity of PG from tomato and cabbage (Choi et al., 1999; Fachin et al., 2004; Verlent et al., 2004b; Verlent et al., 2005). Fachin et al. (2002b, 2004) studied the stability of tomato PG at different temperature–pressure combinations. Purified tomato PG1 and PG2 in 50 mM sodium acetate buffer, pH 4.4, showed very distinct differences of their temperature stability (PG2 is stable up to 55°C, whereas PG1 is stable up to 65°C), but they were found to have very similar pressure stabilities. At 25°C, both isoenzymes were inactivated in the pressure range of 300–500 MPa within 15-minute treatment time. Peeters et al. (2003) also reported that the inactivation of tomato PG1 and PG2 is identical under high-pressure conditions. The heat stable PG1 isoenzyme was still detected in thermally treated (140°C for five minutes) tomato pieces and tomato juice, whereas no

PG1 or PG2 was found in pressure treated (600 MPa/25°C/15 minutes) tomato juice or pieces. This was explained by the dissociation of PG1 under pressure into PG2 and the β-subunit protein and the subsequent pressure inactivation of PG2. The extremely heat resistant β-subunit, prepared from purified PG1 solutions, was capable of combining with PG2 in vitro to generate PG1 (Peeters et al., 2003). Thus, the higher sensitivity of PG1 towards pressure inactivation compared to heat could be explained by its pressure-induced dissociation. Dissociation of oligomeric protein systems occurs at pressures as low as 100 MPa (Crelier et al., 2001)

Other research groups have also demonstrated the susceptibility of PG towards high pressure inactivation in crude extracts, tomato pieces, and juices (Tangwongchai and Ames, 2000; Crelier et al., 2001; Shook et al., 2001; Krebbers et al., 2003). Tangwongchai and Ames (2000) reported that PG is almost totally inactivated after treatment at 500 MPa and 20°C for 20 minutes in a crude aqueous extract (pH 4.4). Crelier et al. (2001) observed a synergistic effect of pressure and temperature on the inactivation of PG in tomato juice at all temperature–pressure conditions investigated (0.1–600 MPa, 30–60°C). Pressure treatment of tomato dices at 600 MPa and 25°C for one minute reduced the PG activity by approximately 60%, whereas after treatment at 800 MPa and 25°C for one minute no PG activity was detectable (Shook et al., 2001). Choi et al. (1999) investigated the effect of a pressure treatment (up to 800 MPa) at 30°C on purified PG from Chinese cabbage. PG dissolved in 20 mM sodium acetate buffer (pH 5.5) was stable up 200 MPa but was slightly inactivated by the application of 400 MPa for one minute. Approximately 70% of the enzyme was inactivated after one-minute treatment at 800 MPa due to a drastic decrease in substrate-binding affinity of the enzyme.

PPO. High pressure inactivation of PPO has been studied in several fruits and vegetables including apples (Anese et al., 1995; Bayindirli et al., 2006), avocados (Lopez-Malo et al., 1998; Weemaes et al., 1998c), banana (Palou et al., 1999; MacDonald and Schaschke, 2000), guava (Lin and Yen, 1998), mango (Guerrero-Beltran et al., 2006), mushrooms (Weemaes et al., 1997b; Matser et al., 2000), peach (Guerrero-Beltran et al., 2005b), pear (Asaka and Hayashi, 1991), strawberry (Dalmadi et al., 2006; Terefe et al., 2010), and white grape (Castellari et al., 1997; Rapeanu et al., 2006). Representative studies in different systems are summarized in Table 2. Many of the PPOs investigated are extremely resistant to high-pressure inactivation (see Table 2). For instance Plum PPO (in phosphate buffer, pH 7.0) was not inactivated by pressures up to 900 MPa at room temperature with inactivation detectable only at 900 MPa and 50°C. Similarly, only slight inactivation of pear PPO (in phosphate buffer, pH 7.0) was observed at 900 MPa and 25°C. However, not all PPOs show such extreme pressure stability. PPOs from apple (Bayindirli et al., 2006), mango (Guerrero-Beltran et al., 2006), white grape (Rapeanu et al., 2006), peach (Guerrero-Beltran et al., 2005b), guava (Lin and Yen, 1995), tomato (Plaza et al., 2003), potato (Gomes and Ledward, 1996), and carrot (Kim et al., 2001) have been found to be relatively less pressure stable (see Table 2). In addition, the more extreme pressure stabilities were reported in model systems at pH values higher than the physiological pH of most fruit and vegetable tissue homogenates (pH $\geq$ 6.0). Lowering the pH usually results in significant sensitization of PPOs towards pressure inactivation, both in model systems and real food systems, which is positive with respect to HPP of fruit and other acidic or acidified products. For instance, the threshold pressure for inactivation of avocado PPO at room temperature decreased from 850 MPa at pH 8.0 to 450 MPa at pH 4.0 (Weemaes et al., 1998a). Lopez-Malo et al. (1998) showed that pressure treatment of acidified avocado-puree (pH 4.1) with 689 MPa reduced PPO activity by 75% and 84% after 10 and 30 minutes holding time, respectively. Such treatment in combination with storage at 5°C significantly improved the stability of the green color component in avocado puree. The natural pH of avocado is around 6. However, guacomolole and other avocado products are acidified. Palou et al. (1999) also observed approximately 75% reduction in PPO activity in acidified banana puree (pH 3.4) after a pressure treatment at 689 MPa for 10 minutes at 21°C. Similar results were obtained for PPOs from other sources (see Table 2).

Synergistic effects of high pressure and antibrowning agents such as ascorbic acid, cystein, benzoic acid, and gluthathione have also been reported. HPP of mango puree for up to 20 minutes at pressure ranging from 379 MPa to 586 MPa resulted only in 25% inactivation of PPO. However, addition of 500 ppm ascorbic acid resulted in about 95.3% inactivation at all studied pressures. Addition of 200 ppm of cystein also improved the level of inactivation at 448 MPa and above and 20-minute treatment resulted in a maximum of 91.6% inactivation (Guerrero-Beltran et al., 2005a). The pressure stability of mushroom PPO was also reported to be decreased in the presence of benzoic acid and glutathione (Weemaes et al., 1997a). On the other hand, addition of 1000 ppm ascorbic acid inhibited the inactivation of PPO in peach puree with "apparent" activation observed up to 316 MPa with 50% increase in activity after 25 minutes at 207 MPa compared to about 20% inactivation under the same condition without ascorbic acid (Guerrero-Beltran et al., 2004).

High pressure-induced increase in PPO activity has been observed both in model systems and tissue homogenates. HPP of crude PPO extract from pear at 100–600 MPa resulted in a significant increase in the activity of the enzyme with a five-fold increase in activity after 10-minute treatment at 500–600 MPa (Asaka and Hayashi, 1991). Similarly, a significant increase in the activity of blueberry PPO extract (pH 3.6) was observed after combined high pressure-thermal processing with a maximum of 4.4-fold increase in activity after 10-minute treatment at 690 MPa and 50°C (Terefe et al., 2009b). Pressure activation of PPO was also reported by other authors for crude PPO extracts from mushroom (Gomes and Ledward, 1996), apple (Anese et al., 1995), and onions (Butz et al., 1994). Increased PPO activity has also been reported after HPP of tomato puree (Plaza et al., 2003), cloudy apple juice (Bayindirli et al., 2006; Buckow et al., 2009), mushroom (Matser et al., 2000), raspberry fruit (Garcia-Palazon et al., 2004), and strawberry puree (Terefe et al., 2010).

Table 2 Summary of representative studies on the effect of high pressure on PPOs from different sources

Source	Medium	Conditions investigated	Effect	Reference
Apple	Phosphate buffer (pH 6.0)	Up to 900 MPa, 25°C	Threshold pressure for inactivation at 25°C, 600 MPa	Weemaes et al. (1998b)
Apple	Extraction buffer			
	pH 4.5	100–900 MPa, 1 min, ambient temperature	Activity increase at 100–300 MPa, 120% increase at 200 MPa, inactivation at 700–900 MPa	Anese et al. (1995)
	pH 7.0		Inactivation at all conditions, complete inactivation at $P \geq 500$ MPa	
Apple (cv. Amasaya)	Cloudy apple juice (pH 3.5)	250–450 MPa, 25–50°C, 0–60 min	~50% activity increase at 450 MPa, 25°C, 15 min; 90% inactivation at 450 MPa, 50°C, 60 min	Bayindirli et al. (2006)
Apple (cv. Boskop)	Cloudy apple juice	0.1–700 MPa, 20–80°C, kinetic	65% activity increase at 400 MPa, 20°C, 5 min. Antagonistic effect at $P^a < 300$ MPa and $T^b \geq 60$°C for pressure–temperature inactivation after initial PPO activation	Buckow et al. (2009)
Avocado	Phosphate buffer (pH 7.0)		Threshold pressure for inactivation at 25°C: 750 MPa, antagonistic effects at $T \geq 62.5$°C and $P \leq 250$ MPa	Weemaes et al. (1998a, 1998b, 1998c)
	Phosphate buffer (pH 8.0)	0.1–900 MPa, 25–77.5°C, kinetic	Threshold pressure: 850 MPa	
	McIlvaine buffer (pH 4)		Threshold pressure: 450 MPa	
Avocado	guacamole (pH 4.3): 1% (w/w) onion, 1.5% (w/w) NaCl, and citric acid	689 MPa, continuous 5–20 min, cyclic: 2–4 five-minute cycles, ambient temperature	78% inactivation at 689 MPa/20 min 85% inactivation: cyclic treatment with four cycles (5 min each)	Palou et al. (2000)
Banana	Banana puree (pH 3.4)	517 MPa and 689 MPa, 21°C, 10 min	Increased activity after 10 min at 517 MPa, 21% inactivation at 689 MPa	Palou et al. (1999)
Banana	Extraction buffer (pH 7.0)	50–139 MPa, 50–70°C, 25 min	Inactivation at all conditions 85% inactivation at 138.6 MPa, 60°C, 15 min	MacDonald and Schaschke (2000)
Blueberry (cv. C97–390)	McIlvaine buffer (pH 3.6)	100–690 MPa, 24–90°C, 10 min	Increased activity at 100 MPa $< P <$ 690 MPa and 30–70°C, and $P = 690$ MPa and 30–50°C; 4.4 times activation at 690 MPa and 50°C; complete inactivation at 90°C	Terefe et al. (2009b)
Broccoli	Broccoli pieces	210 MPa, –20°C and 180 MPa and –16°C (pressure shift freezing)	No inactivation	Prestamo et al. (2004)
Carrot	Carrot juice	100–600 MPa, 25°C, 10 min 300–500 MPa, 50–70°C, 10 min	90% inactivation at 500 MPa and 600 MPa >80% inactivation at 300–500 MPa, 50–70°C	Kim et al. (2001)
Guava	30% Guava juice (pH 4.7, 3° Brix) 30% Guava juice (pH 3.9, 3° Brix) 30% Guava juice (pH 3.9, 12° Brix)	600 MPa, 10 min, 25°C	45% inactivation 70% inactivation at 600 MPa/10 min 50% inactivation	Lin and Yen (1995)
Guava	30% Guava juice (pH 3.9, 12° Brix)	100–500 MPa, 25°C, 5–60 min	16% inactivation at 500 MPa and time ≥ 5 min, no inactivation at $P < 500$ MPa	Lin and Yen (1998)
		300–500 MPa at 10–60°C, 10 min	Antagonistic effect at $P \leq 300$ MPa, $T \geq 40$°C	
		100–500 MPa, 25°C, 15 min continuous and cyclic	No effect of cyclic application	
Mango	Puree (pH 4.5) Puree with 200 ppm L-cystein Puree with 500 ppm L-ascorbic acid	379–586 MPa, 0.03–20 min, 25°C	25% inactivation at all conditions after 20 min 95% inactivation at all conditions At $P \geq 448$ MPa and time ≥10 min: 92% inactivation	Guerrero-Beltran et al. (2005a)

Mushroom	Phosphate buffer (pH 6.8)	100–800 MPa, 1–20 min, ambient temperature	~20% inactivation at 200 MPa, 40% activation at 400 MPa after 10 min, 40% inactivation at 800 MPa	Gomes and Ledward (1996)
Mushroom	Purified PPO in: phosphate buffer (pH 6.5) Phosphate buffer (pH 8.0) McIlvaine buffer (pH 4)	Up to 900 MPa, 25°C	Threshold pressure for inactivation at 25°C 750 MPa 750 MPa 650 MPa	Weemaes et al. (1997a, 1997b)
Mushroom	0.1 M Na-phosphate buffer (pH 6.5)	0.1–500 MPa, –26–20°C, 10 min	36% inactivation (–20°C, 300 MPa, 10 min), slight activation at $P < 300$ MPa and 20°C	Van Buggenhout et al. (2006b)
Mushroom	Mushroom	200–1000 MPa, room temperature, 5 min	52–87% increase in activity at 600 MPa, 65–75% inactivation at 800 MPa	Matser et al. (2000)
Onion (cv. "Stuttgarter Riese")	Tris buffer (pH 6.5)	100–700 MPa, 25°C, 10 min	Activity increase up to a maximum of 42% at 500 MPa	Butz et al. (1994)
Peach	Puree	207–517 MPa, 5–25 min	Inactivation increasing with pressure with 95% inactivation at 517 MPa and 5 min	Guerrero-Beltran et al. (2005b)
	Puree with 1000 ppm ascorbic acid		Increased activity up to 310 MPa (50% at 310 MPa); 80% inactivation at 517 MPa	
	Puree with 300 ppm cystein		100% inactivation at 517 MPa, 5 min	
Pear	Phosphate buffer (pH 7.0)	Up to 900 MPa, 25°C	Slight inactivation at 25°C at 900 MPa	Weemaes et al. (1998b)
Pear (cv. Bartlett)	Crude extract in distilled water	100–600 MPa, 10 min	Activation, 5-fold increase at 400–500 MPa	Asaka et al. (1994); Asaka and Hayashi (1991)
Pear (cv. La France)	Purified latent PPO in phosphate buffer (pH 7.0)	300–700 MPa	Activation at $P > 300$ MPa that increased with treatment time, maximum activation at 600 MPa	
Plum	Phosphate buffer (pH 7.0)	Up to 900 MPa, 25°C	No inactivation at 25°C up to 900 MPa, inactivation at 50°C and 900 MPa	Weemaes et al. (1998b)
Potato	Phosphate buffer (pH 6.8)	100–800 MPa, 1–20 min, ambient temperature	Inactivation at $P \geq 200$ MPa, 60% inactivation at 800 MPa and 10 min	Gomes and Ledward (1996)
Potato		100–500 MPa, –26–20°C		Van Buggenhout et al. (2006b)
	Crude extract	13 min	No inactivation	
	Potato pieces	13 min	No inactivation	
Raspberry	Whole fruit	600–800 MPa/5–15 min	54% and 42% activity increase after 5 min and 10 min at 600 MPa 29% inactivation (800 MPa/15 min)	Garcia-Palazon et al. (2004)
Strawberry	Whole fruit	600–800 MPa/5–15 min	100% inactivation (800 MPa/10 min), ~78% inactivation (600–800 MPa/5 min)	Garcia-Palazon et al. (2004)
Strawberry (cv, Elsanta)	Phosphate buffer (pH 7.0)	0.1–800 MPa, 10–65°C, kinetic	Threshold inactivation pressure at 25°C: 550 MPa, antagonistic effect at $T \geq 50$°C, $P \leq 200$ MPa for the inactivation of the stable fraction (52%)	Dalmadi et al. (2006)

(Continued on next page)

Table 2 Summary of representative studies on the effect of high pressure on PPOs from different sources *(Continued)*

Source	Medium	Conditions investigated	Effect	Reference
Strawberry (cv. Festival)	Strawberry halves	100–600 MPa, 20–60°C, 2–10 min	No significant inactivation	Terefe et al. (2009a, 2010)
Strawberry (cv. Aroma)	Puree	100–690 MPa, 24–90°C, 5–15 min	~16% activity increase at 690 MPa, 24°C, 23% inactivation at 690 MPa and 90°C	
Tomato	Tomato puree:			Plaza et al. (2003)
	Without additives	50–400 MPa, 25°C, 15 min	Activation (50–400 MPa)	
	With 2% (w/w) citric acid, 0.8 (w/w) NaCl		29% inactivation (400 MPa, 15 min)	
White grape (cv. Trebbiano)	Synthetic grape must	300–900 MPa, 2–10 min, ambient temperature	~10% inactivation at 300 and 600 MPa/10 min and 99% inactivation at 900 MPa/10 min	Castellari et al. (1997)
White grapes	McIlvaine buffer (pH 6.0)	Up to 900 MPa, 25°C	Threshold inactivation pressure at 25°C: 700 MPa	Weemaes et al. (1998b)
White grapes (cv. Victoria)	McIlvaine buffer (pH 4.0) enzyme added to synthetic must (pH 4.0)	400–800 MPa, 25°C	Threshold inactivation pressure at 25°C: 600 MPa in all systems	Rapeanu et al. (2006)
	Grape must		70%, 23%, and 14% inactivation in buffer, reconstituted must and grape must, respectively, after 15 min at 800 MPa and 25°C	

[a]*P*: Pressure.
[b]*T*: Temperature.

Substantial degradation of phytochemicals such as anthocyanins (Zabetakis et al., 2000; Kouniaki et al., 2004; Suthanthangjai et al., 2005; Terefe et al., 2009a) have been observed during storage of high pressure-treated products, possibly due to the incomplete inactivation or activation of PPO and other oxidative enzymes.

POD. The sensitivity of PODs towards pressure inactivation varies depending on the source and composition of the medium (see Table 3). For instance, PODs from horseradish and apple (Prestamo et al., 2001) are highly resistant to pressure inactivation with limited inactivation even at pressures as high as 1000 MPa. On the other hand, PODs from guava, carrot, strawberry, and orange have been found to be relatively pressure labile. Lin and Yen (1995) observed 20% inactivation of POD after 10-minute treatment of 30% guava juice (pH 4.7, 3 °Brix) at 600 MPa and ambient condition. Acidifying the juice (pH 3.9) increased the level of inactivation to about 50% while the addition of sugar (12 °Brix) had the opposite effect with inactivation reduced to 30% inactivation. Treatment of the acidified and sweetened juice (pH 3.9, 12 °Brix) at 500 MPa for 10 minutes resulted in 17% inactivation with no further inactivation with increased treatment time, which seems to indicate that the POD in guava consists of isozymes of different sensitivity towards pressure. Such fractional conversion type pressure inactivation has also been observed for POD from apple (Prestamo et al., 2001).

Mild and subambient temperatures have been investigated to enhance the sensitivity of PODs to pressure inactivation with effects dependent on the source and the pressure–temperature range of combinations. Antagonistic effects between pressure and mild heat have been observed in many cases at pressures lower than 500 MPa. Lin and Yen (1998) observed antagonistic effects of mild temperature and pressure on the inactivation of POD in acidified sweetened guava juice (pH 3.9, 12 °Brix) in the pressure range between 100 MPa and 500 MPa and temperatures between 40°C and 60°C. For instance, 10-minute treatment at 500 MPa and 20°C caused 17% inactivation while no effect was observed at the same pressure and 40°C. Similarly, 10-minute treatment at 60°C and atmospheric pressure caused 40% inactivation while only 21% inactivation was observed at 500 MPa and the same temperature (Lin and Yen, 1998). Similar effects were observed for PODs from apple and basil (Trifiro et al., 2006), strawberry (Cano et al., 1997), tomato (Hernandez and Cano, 1998), and orange (Cano et al., 1997). On the other hand, strong synergistic effect of moderately high pressure (70–110 MPa) and mild temperature (50–70°C) was observed in the case of banana POD in cell-free extract where 98.6% inactivation was observed after five-minute treatment at 110 MPa and 70°C (MacDonald and Schaschke, 2000). The inactivation of strawberry POD was also enhanced at higher temperatures at pressure higher than 400 MPa (Terefe et al., 2009a; Terefe et al., 2010). Low temperatures including subzero temperature conditions have limited effect on the stability of PODs towards high pressure (Prestamo et al., 2004; Van Buggenhout et al., 2006b).

Increased POD activity following HPP has been reported in many instances. Treatment of apple pieces for up to 30-minute treatment at pressure ranging from 700 MPa to 1000 MPa did not have a substantial effect on POD, while about a two-fold increase in activity was observed after treatment at 600 MPa, which was attributed to increased enzyme extraction (Prestamo et al., 2001). Similar apparent activation has been observed for PODs from carrot (Anese et al., 1995), tomato (Plaza et al., 2003), strawberry (Garcia-Palazon et al., 2004), and blue berry (Terefe et al., 2009c).

β-glucosidase. The sensitivity of β-glucosidases from different plant sources towards pressure inactivation varies. Garcia-Palazon et al. (2004) studied the effect of high-pressure treatment in the range of 400 MPa to 800 MPa at room temperature on the activity of β-glucosidase in strawberry and raspberry. In red raspberries, only a slight inactivation of about 10% was observed after 15-minute treatment at 600 MPa and 800 MPa. Up to 15-minute treatment at 400 MPa did not have a significant effect on the activity of the enzyme. In contrast, about 76% increase in the activity of β-glucosidase was observed after 15-minute treatment of strawberries at 400 MPa. Treatments at 600 MPa and 800 MPa caused 49% and 61% inactivation after 15-minute treatment, respectively (Garcia-Palazon et al., 2004). Similarly, Zabetakis et al. (2000) observed 50% and 70% increase in the activity of β-glucosidase after 15-minute treatment of strawberries at 200 MPa and 400 MPa, respectively. Treatments at 600 MPa and 800 MPa for the same duration resulted in 50% and 65% inactivation in that order (Zabetakis et al., 2000). Sumitani et al. (1994) measured the effects of high pressure on purified almond β-glucosidase in 0.05 M citrate buffer (pH 5.2). The experiment was conducted to test the hypothesis that improved flavor quality of high pressure-treated peaches was due to the activity of β-glucosidases in peach that remains active after high-pressure treatment. About 15% activity retention was observed with respect to almond β-glucosidase after 10-minute treatment at 400 MPa (Sumitani et al., 1994). Obviously, as the sensitivity of enzymes towards pressure depends both on their origin and environment, no definitive conclusion as to the cause of the improved flavor can be made from such an experiment.

Hamon et al. (1996) studied the effect of combined high pressure and high temperature treatment on β-glucosidases from almond from the point of view of using the enzymes as biocatalysts under high-pressure condition. Studies were conducted on the stability of the enzyme at temperatures up to 90°C and pressures of up to 250 MPa in 0.1 M citrate–phosphate buffer. However, moderate pressures (up to 250 MPa) in combination with mild temperatures inactivated almond β-glucosidase with increasing rates of inactivation at higher temperature and pressure. About 50% inactivation was observed after five-minute treatment at 60°C and 200 MPa (Hamon et al., 1996).

Allinase. Sohn et al. (1996) studied the effects of HPP on allinase activity and its impact on the flavor of garlic. A 10-minute treatment of purified allinase at 300 MPa and 500 MPa resulted in 80% and 100% inactivation of the enzyme, respectively. Similar treatments of garlic cloves resulted in 7% and

Table 3 Summary of representative studies on the effect of high pressure on PODs from different sources

Source	Medium	Treatment condition	Effect	Reference
Apple	Apple pieces	600–1000 MPa, 20°C, 15 and 30 min	Two-fold increase in activity at 600 MPa, ~40% inactivation at 1000 MPa, no effect of treatment time	Prestamo et al. (2001)
Banana	Extraction buffer (pH 7.0)	70–139 MPa, 50–70°C, 5–25 min	98.6% inactivation at 110 MPa and 70°C, 5 min	MacDonald and Schaschke (2000)
Blueberry (cv. C97–390)	McIlvaine buffer (pH 3.6)	100–690 MPa, 24–90°C, 10 min	Complete inactivation at 90°C regardless of the pressure, slight inactivation at 100 MPa and 690 MPa and 30–70°C, maximum 35% inactivation at 690 MPa and 70°C, 40% increase at 400 MPa and 50°C	Terefe et al. (2009c)
Carrot	Phosphate buffer pH 7.0 pH 6.0 pH 5.0	300–900 MPa, 1 min, ambient temperature	Increased activity at 300–500 MPa, highest activation at pH 6.0 followed by pH 7.0, maximum of 70% increase at pH 6.0 and 500 MPa. 600 MPa, 1 min, 40% inactivation at pH 5.0 900 MPa, 1 min, 100% inactivation in all cases	Anese et al. (1995)
Carrot				Van Buggenhout et al. (2006b)
	Crude extract	100–500 MPa, –26°C to 20°C, 13 min	No effect at 100–500 MPa and 20°C, slight inactivation at $T^a \leq -10$°C, maximum of 30% inactivation at –20°C and 500 MPa	
	Carrot pieces		~50% inactivation at 100–200 MPa and 500 MPa and 20°C. ~50% inactivation at 100–200 MPa and –10°C. Limited or no inactivation at other conditions	
Guava	30% Guava juice (pH 4.7, 3° Brix) 30% Guava juice (pH 3.9, 3° Brix) 30% Guava juice (pH 3.9, 12° Brix)	600 MPa, 25°C, 10 min	20% inactivation 50% inactivation 30% inactivation	Lin and Yen (1995)
Guava	30% Guava juice (pH 3.9, 12° Brix)	100–500 MPa, 25°C, 5–60 min	No effect up to 300 MPa, 10.4% inactivation at 500 MPa, ≥5 min,	Lin and Yen (1998)
		300–500 MPa at 10–60°C, 10 min	antagonistic effect (P^b = 100–500 MPa, T = 40–60°C), 21% inactivation at 500 MPa, 60°C/10 min compared to 40% at 0.1 MPa/60°C/10 min and 19% at 500 MPa, 10°C, 10 min.	
		100–500 MPa, 25°C, 15 min: continuous and cyclic	No effect of cyclic pressure application	

Horseradish	Distilled water	600–1000 MPa, 20°C, 15 and 30 min	~25% inactivation at 1000 MPa/15 min	Prestamo et al. (2001)
Horseradish	0.1 M Na-phosphate buffer (pH 7)	100–500 MPa, –26°C to 20°C, 10 min	No inactivation at 0°C and 100–300 MPa, inactivation at –20°C increasing with pressure, maximum of 41.1% inactivation at 300 MPa and –20°C	Van Buggenhout et al. (2006b)
Orange	Orange juice	50–400 MPa, 20–60°C, 15 min	Inactivation at 50–400 MPa and 25–32°C, maximum of 50% inactivation at 400 MPa, 32°C, 15 min, decreased inactivation or activation at higher temperature.	Cano et al. (1997)
Broccoli	Shredded Broccoli	210 MPa, –20°C and 180 MPa and –16°C	No inactivation	Prestamo et al. (2004)
Strawberry	Whole fruit	400–800 MPa/15 min, ambient temperature	13% activity increase at 400 MPa and 5 min, 35% inactivation (600–800 MPa/15 min)	Garcia-Palazon et al. (2004)
Strawberry (cv. Festival)	Strawberry halves puree	100–600 MPa, 20–60°C, 2–10 min	Significant inactivation at all condition, maximum of 58% inactivation at 600 MPa, 60°C, 10 min.	Terefe et al. (2009a, 2010)
Strawberry (cv. Aroma)		100–690 MPa, 24–90°C, 5–15 min	Inactivation at all conditions, almost complete inactivation after 5 min at 90°C regardless of the pressure, 72% inactivation at 690 MPa, 24°C, 15 min. Slight antagonistic effect at $P < 400$ MPa	
Tomato	Tomato puree without additives with 2% (w/w) citric acid	50–400 MPa, 15 min, 25°C	Activation (50–400 MPa) maximum of 37% inactivation (400 MPa, 15 min)	Plaza et al. (2003)

[a]T: Temperature.
[b]P: Pressure.

100% inactivation at 300 MPa and 500 MPa, respectively. The pungency and sulphuryl odor of garlic treated at 500 MPa was greatly reduced due to the inactivation of the enzyme (Sohn et al., 1996).

LOX. Lipoxygenase is one of the enzymes that is relatively more sensitive to high-pressure inactivation compared to other plant enzymes. Substantial inactivation of LOXs from different sources has been reported by HPP, both at ambient temperature and in combination with mild and low temperatures (see Table 4). The threshold pressure for the inactivation of most LOXs is about 300 MPa at ambient temperature. Inactivation at lower pressures usually requires temperature higher than 40°C. Ludikhuyze et al. (1998b) investigated the mechanism of high-pressure inactivation of soybean LOX in Tris-HCl buffer (pH 9) by measuring the activity of the enzyme spectrophotometrically and by following the change in the intensity of the major band in the electropherogram of gel electrophoresis following HPP. Treatment in the pressure range of 450 MPa to 600 MPa caused a significant change in the activity of the enzyme while no significant change was observed in the intensity of the band in the electropherogram, indicating that the inactivation of the enzyme was not accompanied by complete denaturation. At pressures between 600 MPa and 800 MPa, a significant change in the intensity of the band and hence denaturation of the enzyme was observed. The study showed that at pressures lower than 600 MPa, minor conformational changes of the active site may have caused loss of activity without a significant change in the conformation of the enzyme (Ludikhuyze et al., 1998b). Likewise, Fourier transform infrared spectroscopy (FTIR) studies of soybean LOX have shown that high-pressure treatment in the range of 600 MPa to 1200 MPa causes changes in the amide I band, which correlates to structural changes of the enzyme as well as loss of activity (Heinisch et al., 1995).

Compared to other food enzymes, LOXs are relatively more sensitive to pressure at subambient temperature conditions. Indrawati et al. (1998) studied the sensitivity of a number of food enzymes including soybean LOX towards high pressure-assisted freezing and compared it with classical freezing. Soybean LOX was found to be more sensitive to high pressure-low temperature treatment than all the other enzymes studied, which included purified horseradish POD, orange peel PME, and mushroom PPO. The inactivation of soybean LOX in Tris-HCl buffer (pH 9) increased with increasing pressure and decreasing temperature. Almost complete and irreversible inactivation of the enzyme was observed after a treatment at 400 MPa and –20°C for 10 minutes. Conventional freezing caused about 10% inactivation and further 10% inactivation was observed during three hours of storage at 0°C (Indrawati et al., 1998). Likewise, increased sensitivity towards pressure inactivation of LOXs from tomato (Rodrigo et al., 2006b; Van Buggenhout et al., 2006b), green beans (Indrawati et al., 2000), and green peas (Indrawati et al., 2001) has been observed at subambient temperature conditions. A common peculiar feature of LOXs from these different sources is the occurrence of maximum pressure stability at temperatures between 10°C and 30°C. Maximal pressure stability of soybean LOX and tomato LOX was observed around 30°C (Ludikhuyze et al., 1998c; Indrawati et al., 1999b; Rodrigo et al., 2006b) whereas maximum pressure stability of green beans and green peas LOX was observed at 10°C (Indrawati et al., 2000; Indrawati et al., 2001). In all cases, antagonistic effects of pressure and temperature were observed at temperatures less than the temperature of maximum stability.

On the other hand, temperature increase above the temperature of maximum stability has been found to enhance the rate of inactivation of LOXs at all pressure–temperature combinations except in the case of tomato and green peas LOX, where an antagonistic effect was observed at moderately high pressure and high temperature conditions (see Table 4). Possibly, this allows HPP at moderate pressure and temperature conditions without significant effect on the quality of the product. Kim et al. (2001) studied the effect of combined high pressure-mild temperature (100–600 MPa, 25–70°C) processing on the activity of quality-related enzymes in carrot juice including LOX. The effect of high pressure was also compared to standard thermal processing of carrot juice—a blanching pre-treatment (30 seconds in boiling 0.05 N acetic acid solution) followed by treatment of the juice at 105°C for 30 seconds. Combined high pressure-mild temperature processing had more effect on LOX than the treatment at ambient condition. At 25°C, the highest inactivation of 83% occurred at 300 MPa. Treatments at 500 MPa and temperatures of 60°C to 70°C resulted in almost complete inactivation of carrot LOX. In all cases, high-pressure inactivation was higher than that achieved by thermal processing, which was 54%. Compared to thermal processing, better retention of α-carotene and β-carotene, respectively, were observed after combined high pressure-mild temperature processing, although the difference between the two decreased with increase in temperature (Kim et al., 2001).

HPL. There are not many studies on the effect of HPP on HPL. Rodrigo et al. (2007) studied the high-pressure inactivation of tomato HPL in tomato juice at 20°C. High-pressure treatment at pressures above 200 MPa caused inactivation of HPL with 12-minute treatment at 300 MPa and 550 MPa causing 20% and 80% inactivation, respectively. No further increase in the inactivation was observed up to 650 MPa. At pressures lower than 400 MPa, HPL was found to be more pressure sensitive than LOX while the opposite was observed at pressures higher than 400 MPa (Rodrigo et al., 2007).

Myrosinase. Ludikhuyze et al. (1999) studied the effects of combined high pressure thermal treatment on partially purified broccoli myrosinase in 0.1 M phosphate buffer (pH 6.55), which corresponded to the pH of the broccoli juice. The high-pressure inactivation of the enzyme was studied in the pressure range between 350 MPa and 500 MPa at 20°C and at pressure ranging between 0.1 MPa and 450 MPa at 35°C. Pressures lower than 250 MPa did not have any effect on the enzyme at 20°C. However, significant inactivation of the enzyme was observed at pressures between 300 MPa and 500 MPa (Ludikhuyze et al., 1999). The inactivation kinetics of the enzyme was also studied in broccoli juice at temperatures between 10°C and 60°C and

Table 4 Summary of representative studies on the effect of high pressure on LOXs from different sources

Source	Medium	Treatment condition	Effect	Reference
Avocado	Guacamole (pH 4.3): 1% (w/w) onion, 1.5% (w/w) NaCl, and citric acid	689 MPa, continuous 5–20 min, cyclic: 2–4 five-min cycles, ambient temperature	95% inactivation at 689 MPa/10 min continuous. 100% inactivation: two 5-min cycles	Palou et al. (2000)
Carrot	Carrot juice	100–600 MPa, 25°C, 10 min	Maximum 83% inactivation at 300 MPa, 10 min, 25°C. ~65% inactivation at $P^a > 300$ MPa.	Kim et al. (2001)
		300–500 MPa, 50–70°C, 10 min	Complete inactivation at $P = 500$MPa, $T^b = 60$–70°C	
Green beans	Green beans juice	500 MPa, 20°C, 10 min	50% inactivation at 500 MPa/20°C/10 min.	Indrawati et al. (1999a, 2000)
	Green beans juice	0.1–650 MPa, –10°C to 70°C, kinetic	Antagonistic effect at $P \geq 400$ MPa, $-10 \leq T \leq 10$°C, lower stability in intact tissue	
	Intact green beans			
Green peas	Green peas juice	0.1–625 MPa, –15°C to 70°C, kinetic	Antagonistic effects at $P \leq 650$ MPa and $10 \leq T \leq 10$°C and $T \geq 60$°C and $P \leq 200$MPa	Indrawati et al. (2001)
	Intact green peas			
Soybean	Tris-HCl buffer (pH 9)	0.1–400 MPa, –22°C to 0°C	100% inactivation (400MPa/–20°C/10 min) compared to 40% after classical freezing (–18°C) for 2 hours.	Indrawati et al. (1998, 1999b)
		0.1–650 MPa, –15°C to 68°C, kinetic	Antagonistic effect at 300–650 MPa, $-15 \leq T \leq 30$°C, at $P \leq 250$ MPa, $T \geq 45$°C required for inactivation	
Soybean	Tris-HCl buffer (pH 9)	525 MPa, 10–25°C, kinetic	Substantial decrease in pressure stability in McIlvaine buffer compared to Tris buffer.	Ludikhuyze et al. (1998a)
	Tris-HCl buffer (pH 6.6)	400 MPa, 10–25°C	Accelerated inactivation at lower pH in both buffers.	
	McIlvaine buffer (pH 6.6)	400 MPa, 10–25°C	2–10 times higher inactivation rate by CO_2 flushing compared to Tris-HCl buffer at pH 6.6	
	McIlvaine buffer (pH 5.4)			
	Tris-HCl buffer (pH 9) flushed with CO_2: final pH ~6.8			
Tomato	Extraction buffer	100–500 MPa, –26°C to 20°C, 13 min	>60% inactivation at $P \geq 300$ MPa, –26°C to 20°C, ~90% inactivation at 500 MPa/20°C.	Van Buggenhout et al. (2006b)
	Tomato pieces		No inactivation at 20°C at $P < 500$ MPa and ~10% inactivation at 500 MPa, Inactivation at $P \geq 300$ MPa, –20°C to –10°C, complete inactivation at 400–500 MPa, –10°C to –20°C, limited effect at $T = -26$°C up to 500 MPa	
Tomato	Partially purified enzyme reconstituted into tomato puree (20° Brix)	0.1–650 MPa, 10–60°C, kinetic	53% inactivation at 550 MPa/20°C/12 min. Antagonistic effect at $T \geq 50$°C and $P \leq 300$ MPa and $10 \leq T \leq 30$°C, $P \geq 400$ MPa	Rodrigo et al. (2006b)
Tomato	Tomato juice (4° Brix)	100–650 MPa, 20°C, 12 min	Increased activity at 400 MPa, complete inactivation at 550 MPa, 20°C/12 min	Rodrigo et al. (2007)

[a]P: Pressure.
[b]T: Temperature.

pressure of 100 MPa to 600 MPa (Van Eylen et al., 2007). At temperatures higher than 50°C, antagonistic effects of temperature and pressure were observed at pressure up to 200 MPa, with an increase in pressure slowing down the rate of inactivation. For instance, 97% inactivation of the enzyme occurred after 20-minute treatment at 60°C, while only 75% inactivation occurred at 100 MPa and the same temperature condition. Interestingly, 77% of the inactivation occurred during the pressure build up (Van Eylen et al., 2007). A subsequent study in broccoli tissue showed similar susceptibility of the enzyme to pressure inactivation with antagonistic effects between pressure and temperature observed at temperatures higher than 50°C and pressure ≤150 MPa. For example, 15-minute treatment at 60°C and atmospheric pressure resulted in 95% inactivation of the enzyme whereas the inactivation at the same condition and 100 MPa was only 45% (Van Eylen et al., 2008b).

The same group (Van Eylen et al., 2006) investigated the high pressure–temperature stability of partially purified mustard seed myrosinase in 20 mM Bis-Tris buffer (pH 6.5) at pressures ranging from 100 MPa to 800 MPa and temperatures of 10°C to 70°C. Interestingly, mustard seed myrosinase was found to be quite pressure stable. Ten-minute treatments at pressures up to 600 MPa and temperatures up to 70°C did not have any effect on the activity of the enzyme, while a significant thermal inactivation of the enzyme occurred at 70°C under atmospheric pressure. Even at higher pressure conditions, the inactivation of the enzyme was very slow. High pressure treatment at 50°C and 750 MPa for 50 minutes caused only 20% inactivation of the enzyme (Van Eylen et al., 2006). The pressure stability of the enzyme was also investigated in broccoli juice where the endogenous myrosinase was treated at 40–60°C and pressures between 0.1 MPa and 700 MPa. No inactivation of the enzyme was observed in the whole pressure–temperature domain (Van Eylen et al., 2008a).

High-Pressure Inactivation Kinetics of Enzymes

There are several kinetic studies on the combined high pressure–temperature inactivation of plant enzymes, mainly in model systems. Detailed inactivation kinetics studies have been reported for PMEs, PGs, PPOs, LOXs, and myrosinases from various sources. As in the case of thermal inactivation kinetics, the inactivation kinetics of enzymes under high-pressure conditions often follows first-order model (Equation (1)):

$$A = A_o \exp(-kt), \tag{1}$$

where A is enzyme activity at time t, A_o is initial enzyme activity, and k is the first-order inactivation rate constant in [min^{-1}]

In food processing, first-order reactions are commonly described by D values. The decimal reduction time D is the time for 90% reduction in activity A under constant temperature and pressure conditions. It can be estimated from the slope of log (A/A_o) versus time curve as described in Equation (2):

$$\log\left(\frac{A}{A_o}\right) = -\frac{t}{D}. \tag{2}$$

In cases when several isoenzymes of different stability exist together, the inactivation kinetics may be described by either the biphasic (Equation (3)) or the fractional conversion (Equation (4)) models, which are special forms of first-order kinetic model:

$$A = A_s \exp(-k_s t) + A_L \exp(-k_L t), \tag{3}$$

where A_s and A_L are activities of the stable and the labile fractions, respectively, and k_s and k_L are the inactivation rate constants of the stable and the labile fractions, respectively.

The fractional conversion model applies when there is a residual enzyme fraction whose activity remains constant after prolonged treatment time (Van den Broeck et al., 1999):

$$A = A_o + (A_o - A_\infty) \exp(-kt), \tag{4}$$

where A_∞ is the residual activity after prolonged treatment time, i.e., the activity of the stable fraction.

First-order inactivation kinetic models have been successfully used to describe the combined high pressure thermal inactivation kinetics of enzymes including PMEs from tomato (Crelier et al., 2001), grapefruit (Guiavarc'h et al., 2005), plum (Nunes et al., 2006), and carrot (Balogh et al., 2004); PG from tomato (Fachin et al., 2002b, 2003, 2004); PPOs from avocado (Weemaes et al., 1998c), mushroom (Weemaes et al., 1997a), and white grape (Rapeanu et al., 2006); and LOXs from soybean (Indrawati et al., 1999b), green peas (Indrawati et al., 2001), and tomato (Rodrigo et al., 2006b). Fractional conversion and biphasic inactivation kinetics are usually observed for purified enzyme extracts where isoenzymes of different stability were not fully separated or in cases where the inactivation kinetics of the enzymes were investigated in their natural medium. Fractional conversion kinetics were reported in the case of PMEs from orange (Van den Broeck et al., 1999), banana (Ly-Nguyen et al., 2003b), carrot (Ly-Nguyen et al., 2002b, 2002c), strawberry (Ly-Nguyen et al., 2002c), grape fruit (Guiavarc'h et al., 2005), and capsicum (Castro et al., 2006b) in model systems and orange PME in orange juice (Goodner et al., 1998; Nienaber and Shellhammer, 2001b). Biphasic inactivation kinetics were observed for PPOs from white grape in grape must (Rapeanu et al., 2006) and strawberry (Dalmadi et al., 2006). Reported pressure inactivation kinetic parameters of quality-related enzymes at relevant conditions are summarized in Table 5 together with corresponding thermal inactivation parameters. As can be seen, under economically feasible and practical conditions, thermal pasteurization (up to a few minutes at temperature less than 100°C) is more effective than HPP (1–5 minutes at 600 MPa) for inactivating quality-degrading enzymes in fruit and vegetable products.

Table 5 High pressure and thermal inactivation kinetic parameters of quality related enzymes from different sources

Enzyme	Medium	$D_{thermal}$ [min]	D_{HPP} [min]	Reference
Navel orange PME (thermolabile)	Citric acid buffer (pH 3.7)	$D_{65} = 2.2$	$D_{600,25} = 58.3$ $D_{600,45} = 34.07$	Van den Broeck et al. (2000a)
Orange peel PME (thermolabile)	Deionized water (pH 4.5)	$D_{70} = 0.55$	$D_{600,45} = 44.4$	Van den Broeck et al. (2000a)
Orange peel PME (thermostable)	Deionized water (pH 4.5) Citric acid buffer (pH 3.7)	$D_{82} = 1.86$ $D_{75} = 62.2$ $D_{73} = 6.3$		Van den Broeck et al. (2000a)
Orange PME (thermolabile)	Florida orange juice		$D_{600,25} = 7.0$ $D_{600,50} = 4.6$	Nienaber and Shellhammer (2001b)
Tomato PME	50 mM citrate buffer (pH 4.4) Tomato juice	$D_{70} = 3.08$ $D_{71} = 1.2$	No inactivation up to 700 MPa	Fachin et al. (2002a)
Tomato PME	Tomato juice	$D_{70} = 6.1$	$D_{600,60} = 876.3$	Crelier et al. (2001)
White grape fruit PME (thermolabile)	20 mM Tris buffer (pH 7)	$D_{70} = 0.4$	$D_{600,30} = 135.2$ $D_{600,58} = 5.7$	Guiavarc'h et al. (2005)
Carrot PME (thermolabile, thermostable)	20 mM Tris-HCl buffer (pH 7.0)	$D_{60} = 2.65$ $D_{66} = 4.85$	$D_{600,30} = 307$ $D_{600,65} = 29.2$ No inactivation to 825 MPa	Ly-Nguyen et al. (2003a)
Purified carrot PME Endogenous carrot PME	0.1 M citrate buffer (pH 6) pH 4.5 Carrot juice (pH 6.0) Carrot pieces	$D_{60} = 3.59$ $D_{56} = 3.49$ $D_{60} = 7.32$ $D_{66} = 648.5$ $D_{74} = 4.13$	$D_{700,25} = 161.3$ $D_{700,10} = 188$ $D_{650,25} = 72.6$ $D_{700,10} = 171.6$ $D_{700,40} = 391.5$	Balogh et al. (2004)
Banana PME (thermolabile) Thermostable PME	20 mM Tris-HCl buffer (pH 7)	$D_{70} = 41.9$ $D_{76} = 3.65$	$D_{600,70} = 247.6$ $D_{600,76} = 116.9$ Not inactivated up to 900 MPa	Ly-Nguyen et al. (2003b)
Pepper PME (thermolabile, thermostable)	Citrate buffer (pH 5.6)	$D_{64} = 2.9$	$D_{600,25} = 103.7$ $D_{600,56} = 17$ No inactivation up to 800 MPa	Castro et al. (2006b)
Strawberry PME (thermolabile)	20 mm Tris-HCl buffer (pH 7.0)	$D_{63} = 4.66$	$D_{850,10} = 175.8$	Ly-Nguyen et al. (2002c)
Plum PME	20 mM Tris buffer (pH 7.5)	TL: $D_{70} = 1.2$ (59%) TS: $D_{70} = 6.9$ (41%)	$D_{650,25} = 68.5$	Nunes et al. (2006)
Tomato PG	Tomato juice	D80 = 85 D90 = 20	$D_{500,30} = 9.7$ $D_{600,40} = 22.3$	Crelier et al. (2001)
Tomato PG	Tomato juice	PG2: D70 = 3 (86% PG2)	$D_{500,25} = 5.2$	Fachin et al. (2003)
Purified tomato PG2	50 mM Na-acetate buffer (pH 4.4)	D63 = 1.5	$D_{500,20} = 15.2$ $D_{600,10} = 12.7$ (PG1 same)	Fachin et al. (2004)

(Continued on next page)

Table 5 High pressure and thermal inactivation kinetic parameters of quality related enzymes from different sources *(Continued)*

Enzyme	Medium	$D_{thermal}$ [min]	D_{HPP} [min]	Reference
Avocado PPO	0.1 M phosphate buffer (pH 7)	$D_{62.5} = 343.7$ $D_{77.5} = 2.06$	$D_{750,25} = 2559$ $D_{600,62.5} = 118.7$ $D_{600,72.5} = 31.3$	Weemaes et al. (1998c)
Purified mushroom PPO	Phosphate buffer (pH 6.5)	$D_{55} = 32$	$D_{550,55} = 96$	Weemaes et al. (1997b)
Purified white grape PPO (var. victoria) Endogenous	McIlvaine buffer (pH 4.0) Grape must (pH 4.0)	$D_{60} = 3.83$ TL[a]: $D_{60} = 0.96$ (49.7%)	$D_{600,25} = 65.7$ TS: $D_{600,25} = 204.8$	Rapeanu et al. (2006)
White grape PPO	Grape must (pH 4.4)	TS[b]: $D_{60} = 15.2$ (50.3%) TL: $D_{60} = 17.1$ (58.9%) TS: $D_{60} = 299.1$ (41.1%)	TS: $D_{600,25} = 370.3$	
Purified apple PPO	Clarified apple juice pH 3.0 pH 3.5		 $D_{400,30} = 31$ $D_{400,30} = 73$	Ramaswamy and Riahi (2003)
Purified strawberry PPO	0.1 M phosphate buffer (pH 7.0)	TL: $D_{60} = 1.9$ TS: $D_{60} = 14.4$	TS: $D_{600,25} = 324.3$ $D_{600,50} = 38.9$	Dalmadi et al. (2006)
Soybean LOX	0.01 M Tris-HCl buffer (pH 9.0)	$D_{68} = 12.6$	$D_{600,15} = 14.3$ $D_{600,25} = 40$	Indrawati et al. (1999b)
Green beans LOX	Green bean juice Intact green beans	$D_{68} = 5.8$ $D_{68} = 6.8$	$D_{600,0} = 26.8$ $D_{600,30} = 60$ $D_{450,50} = 28$ $D_{550,30} = 16.2$ $D_{450,50} = 33.6$	Indrawati et al. (2001)
Green peas LOX	Green pea juice Intact green peas	$D_{70} = 2.9$ $D_{70} = 3.6$	$D_{600,20} = 23.9$ $D_{500,0} = 30.7$ $D_{500,20} = 18$ $D_{500,0} = 38.7$	Indrawati et al. (2001)
Purified tomato LOX	Tomato puree	TL: $D_{65} = 0.4$ (85.7%) TS: $D_{65} = 2.9$ (14.3%)	$D_{600,10} = 6.6$ $D_{600,20} = 9.0$	Rodrigo et al. (2006b)

[a]TL: thermolabile.
[b]TS: thermostable.

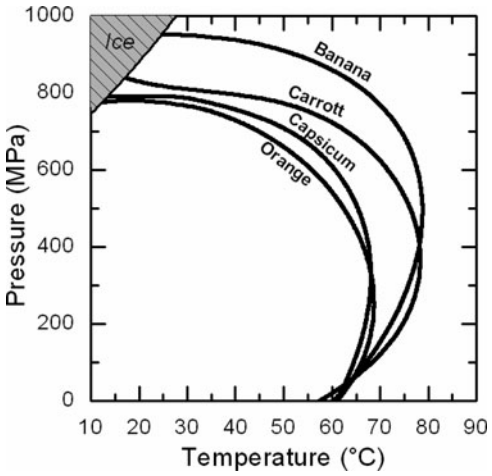

Figure 1 Pressure–temperature isorate diagram for 1 log inactivation of PME from orange (in 5 mM citric acid buffer, pH 3.7; Van den Broeck et al., 2000a), capsicum (in 20 mM citrate buffer, pH 5.6; Castro et al., 2006a), carrot (in 20 mM Tris buffer, pH 7.0; Ly-Nguyen et al., 2003a), and banana (in 20 mM Tris buffer, pH 7.0; Ly-Nguyen et al., 2003c), respectively, after 10 minutes isothermal/isobaric treatment.

In combined high pressure thermal inactivation, many enzymes display elliptical contour diagrams as shown in Figs 1 and 2 for PMEs and PPOs from different sources. These contour diagrams depict an antagonistic effects between pressure and temperature at moderate pressure ($P < 400$ MPa) and moderately high temperature ($>50^{\circ}$C), whereas synergistic effects are observed at all other conditions. The term synergistic in this case is used as opposed to antagonistic. In reality, close examination of the published inactivation data reveals that the combined effect of pressure and temperature is additive rather than synergistic in the actual sense of the term. Antagonistic effects between moderate pressures ($P < 400$ MPa) and temperatures

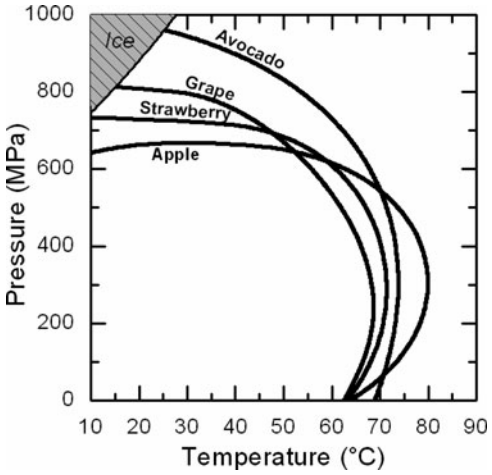

Figure 2 Pressure–temperature isorate diagram for 1 log inactivation of PPO from apple (in cloudy apple juice, pH 3.6; Buckow et al., 2009), strawberry (in 0.1 M phosphate buffer, pH 5.0; Dalmadi et al., 2006), white grape (in Victoria grape must, 13.34 Brix, pH 4.03; Rapeanu et al., 2005), and avocado (in 0.1 M phosphate buffer, pH 4.0; Weemaes et al., 1998c), respectively, after 10 minutes isothermal/isobaric treatment.

($T \geq 50^{\circ}$C), where pressure inhibits thermal inactivation, have been reported for many enzymes including PMEs from orange (Van den Broeck et al., 2000a; Polydera et al., 2004), grape fruit (Guiavarc'h et al., 2005), banana ((Ly-Nguyen et al., 2003b), capsicum (Castro et al., 2006b), and carrot ((Ly-Nguyen et al., 2003a); PPOs from strawberry (Dalmadi et al., 2006), grape (Rapeanu et al., 2005), and avocado (Weemaes et al., 1998c); PODs from strawberry (Terefe et al., 2009a); LOXs from green peas (Indrawati et al., 2001) and tomato (Rodrigo et al., 2006b); and myrosinases from broccoli (Van Eylen et al., 2007, 2008b) and mustard seed (Van Eylen et al., 2006). However, exceptions have been noted in the case of tomato PG (Crelier et al., 2001; Fachin et al., 2002b, 2004) and LOXs from soybean (Ludikhuyze et al., 1998c) and green beans (Indrawati et al., 2000), where no such antagonistic effect was observed. Increase in pressure at fixed temperature led to an increased rate of inactivation of those enzymes in the entire pressure–temperature domain studied. On the other hand, for LOXs from soybean (Indrawati et al., 1999b), green peas (Indrawati et al., 2001), green beans (Indrawati et al., 2000), and tomato (Rodrigo et al., 2006b), antagonistic effect was observed in the elevated pressure-low temperature domain where an increase in temperature in the low temperature range (subzero to 30°C) resulted in decreased inactivation rates to a minimum value followed by an increase with further increase in temperature.

The Arrhenius equation (Equation (5)) and the Eyring equation (Equation (6)) are commonly used to describe the temperature and pressure dependence, respectively, of the inactivation of enzymes during combined high pressure-thermal processing. The Arrhenius equation can be written as

$$\ln(k) = \ln(k_{\text{ref}}) - \frac{E_A}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right), \tag{5}$$

where T and T_{ref} are the experimental and reference temperatures (K), E_A is the activation energy (J/mol), R is the universal gas constant (8.314 J/mol.K), and k and k_{ref} are the inactivation rate constants (s^{-1}) at T and T_{ref}, respectively. The Eyring equation can be written as

$$\ln(k) = \ln(k_{\text{Pref}}) - \frac{V_a}{RT}(P - P_{\text{ref}}), \tag{6}$$

where P and P_{ref} are experimental and reference pressures, respectively, V_a the activation volume, and k and k_{Pref} are the inactivation rate constants at P and P_{ref}, respectively. From the slopes of the semi-logarithmic plots of the observed rate versus the reciprocal of the absolute temperature and pressure, the activation energy (E_A) and the activation volume (V_a) can be obtained respectively, which characterize the effects of temperature and pressure on the rate of inactivation. The higher the activation energy, the higher is the temperature dependence of the inactivation rate at a given pressure. Similarly, the higher the absolute value of the activation volume, the higher the pressure dependence of the inactivation rate, with negative activation volumes indicating

that inactivation is favored by increase in pressure and vice versa. The Arrhenius equation has been successfully used to describe the temperature dependence of inactivation of enzymes at elevated pressure, including PMEs from orange (Van den Broeck et al., 2000a; Polydera et al., 2004), grapefruit (Guiavarc'h et al., 2005), and pepper (Castro et al., 2006b); tomato PG in tomato juice (Fachin et al., 2003) and buffer (Fachin et al., 2004); and PPOs from avocado (Weemaes et al., 1998c) and strawberry (Dalmadi et al., 2006). Due to the observed antagonistic effect of pressure and temperature in the low temperature-high pressure domain, the Arrhenius equation failed to describe the inactivation of LOXs from various sources including soybean (Indrawati et al., 1999b), green beans (Indrawati et al., 2000), green peas (Indrawati et al., 2001), and tomato (Rodrigo et al., 2006b).

For the majority of enzymes investigated including PMEs and PPOs from various sources, LOXs from green peas (Indrawati et al., 2001) and tomato (Rodrigo et al., 2006b), and broccoli myrosinase, the Eyring equation did not describe the pressure dependence of the inactivation rates in the entire pressure–temperature domain due to the antagonistic effects of pressure and temperature in the moderate pressure-mild temperature range. On the other hand, the Eyring equation has been successfully used to describe the pressure dependence of the inactivation of enzymes including purified tomato PG2 (Fachin et al., 2004) and LOXs from soybean (Indrawati et al., 1999b) and green beans (Indrawati et al., 2000) in the entire pressure–temperature range.

A number of empirical and semi-empirical secondary models have also been proposed to describe the combined pressure–temperature inactivation contours of enzymes as well as micro-organisms. The most commonly used equations are the thermodynamic-based equation (Equation (7)) and the polynomial equation (Equation (8)).

Equation (7) is based on the thermodynamic model originally proposed by Hawley (Hawley, 1971) converted into a kinetic model using Eyring's transition state theory (Ludikhuyze et al., 2003). It can be written as

$$\ln(k) = \ln(k_o) - \frac{\Delta V_o^{\neq}}{RT}(P - P_o) + \frac{\Delta S_o^{\neq}}{RT}(T - T_o) - \frac{1}{2}\frac{\Delta \kappa^{\neq}}{RT}(P - P_o)^2 - \frac{\Delta \zeta^{\neq}}{RT}(P - P_o)(T - T_o), + \frac{\Delta C_p^{\neq}}{RT}\left[T\left(\ln\frac{T}{T_o} - 1\right) + T_0\right] \quad (7)$$

where T_o and P_o are reference temperature (K) and pressure (MPa), respectively; ΔV and ΔS are changes in molar volume (cm^3/mol) and entropy (J/mol.K) between the native and the denatured state, respectively; C_p is heat capacity (J/mol.K); ζ is thermal expansibility coefficient (cm^3/mol.K); and κ is compressibility factor (cm^6/J.mol).

The thermodynamic equation has been used to describe the pressure–temperature dependence of soybean LOX (Indrawati et al., 1999b), green peas LOX in juice and intact tissue (Indrawati et al., 2001), green beans LOX in juice and intact tissue (Indrawati et al., 2000), and broccoli myrosinase in juice and intact tissue after adding a third-order pressure term. The equation in its modified form that uses a second-order approximation of the logarithmic term with addition of third-degree terms has been successfully used to describe the combined high pressure thermal inactivation kinetics of PMEs from carrot (Ly-Nguyen et al., 2002b, 2002c), banana (Ly-Nguyen et al., 2003b), grapefruit (Guiavarc'h et al., 2005), and capsicum (Castro et al., 2006b) and for strawberry PPO (Dalmadi et al., 2006) without the addition of third-order terms. The thermodynamic equation, with modification to eliminate the second-order pressure term and the logarithmic term, has been used to describe the pressure–temperature inactivation of tomato PG in crude extract (Fachin et al., 2002b) and juice (Fachin et al., 2003), where the contour plots were not fully elliptical.

The polynomial equation can be written as:

$$\log(k) = a + b(P - P_o) + c(T - T_o) + d(P - P_o)^2 + e(P - P_o)(T - T_o) + f(T - T_o)^2, \quad (8)$$

where a, b, c, d, e, and f are empirical constants. Although it is purely empirical, the polynomial equation is of similar form as the thermodynamic equation and is commonly used for complex systems where the thermodynamic equation is not applicable. The polynomial equation as is, or with modifications to include higher-order terms, has been used to describe the pressure–temperature dependence of inactivation of enzymes including PMEs from banana (Ly-Nguyen et al., 2003b), PPO from apple (Buckow et al., 2009), and LOX from tomato (Rodrigo et al., 2006b).

Other approaches use either Arrhenius or Eyring equations as a starting point and empirical relations relating E_A and k_{ref} with pressure or V_a and k_{refp} with temperature. The former has been used to model the pressure–temperature dependence of the inactivation rate of PMEs from orange (Van den Broeck et al., 2000a), PPO from avocado (Weemaes et al., 1998c), and the latter for LOX from soybean (Ludikhuyze et al., 1998a) with success. Nevertheless, extrapolation of models based on this approach outside the experimental temperature–pressure domain has been reported to lead to inflection or discontinuity of the isorate diagrams, whereas no such problems are encountered with the thermodynamic and the polynomial model (Ludikhuyze et al., 2003).

Factors That Affect the Sensitivity of Enzymes to Pressure Inactivation

Based on their study of a number of food enzymes in model systems, Seyderhelm et al. (1996) ranked the sensitivity of the enzymes towards pressure inactivation from the most sensitive

to the least sensitive in the following order: LOX, lactoperoxidase, PME, lipase, alkaline phosphatase, catalase, PPO, and POD (Seyderhelm et al., 1996). Nevertheless, such rankings are too simplistic, since the stability of enzymes and their susceptibility towards inactivation either by high pressure or other physical agent depends on intrinsic factors such as the source of the enzyme, pH, and medium composition. From the forgoing discussion, it is clear that not all enzymes have similar susceptibility to pressure induced inactivation. Among plant enzymes, PME, PPO, and POD are often highly resistant to pressure inactivation whereas PGs and LOXs are relatively more susceptible. Nevertheless, the stability of enzymes have been found to be highly dependent on their source making stability ranking solely based on the type of enzyme impractical. Extrinsic factors such as temperature also play a significant role on the susceptibility of enzymes towards high pressure-induced inactivation. The mode of pressure application (continuous versus cyclic) has also been shown to have an effect on the inactivation of some enzymes, although this is basically due to the dynamic condition created by the compression–decompression cycle, affecting the temperature history of the product and hence the enzyme inactivation kinetics.

Origin. One of the factors that affect the susceptibility of enzymes towards high-pressure inactivation is the source or the origin of the enzyme. As can be seen in Tables 1–4, the same types of enzymes from different sources greatly differ in their stability towards pressure inactivation. For instance, PMEs from different sources widely differ in their susceptibility towards pressure inactivation (see Table 1). The same applies to other enzymes. The inactivation rate of enzymes may also depend on the variety, growing condition, and degree of ripening with different isoenzymes present in different proportions in different varieties and at different stages of ripening. There are not many studies addressing this issue. However, the variation in the reported inactivation kinetic parameters of the same types of enzymes from similar sources in literature can be attributed to differences in variety and growing conditions. On the other hand, investigations of purified tomato PME from different varieties did not show any difference in their pressure and temperature stabilities. Similar results were observed for purified PG from the same sources (Rodrigo et al., 2006a).

Matrix composition. The stability of enzymes in simple buffer solutions is clearly different to complex food matrices. In general, an enzyme is more stable in an intact tissue or in a tissue homogenate, where it is protected by the presence of other materials such as proteins and carbohydrates, than in its purified form (Whitaker, 1972). The inactivation of purified carrot PME was studied in different model systems (0.02 M Tris buffer at pH 6.5 and pH 7.0; 0.1 M citrate buffer at pH 4.5, 5.5, and 6.0), carrot juice, and tissue (Balogh et al., 2004). In carrot tissue, PME was much more heat stable and pressure stable than in carrot juice or in purified form. At 800 MPa and 40°C, the *D* value of PME in carrot tissue was 75.8 minutes, whereas at 800 MPa and 10°C it was just 36.2 minutes, and 30.7 minutes for PME in carrot juice and buffer solution (pH 6.0), respectively (Balogh et al., 2004). Similarly, tomato LOX was found to be more sensitive to pressure inactivation in cell-free extract compared to tomato pieces, tomato juice, and tomato puree (Van Buggenhout et al., 2006b; Rodrigo et al., 2007).

The opposite has been reported in some cases where the same or higher rate of pressure inactivation of enzymes was reported in intact tissue or tissue homogenates compared to model systems. In the study of Crelier et al. (2001) on tomato PME, no difference was observed in the pressure inactivation rate of PME in tomato juice (pH 4.2) and in 0.5 M NaCl enzyme extraction solution (pH 4.2) at 300 MPa and 800 MPa and different temperature conditions. A study by Castro et al. (2005) showed that purified capsicum PME in citrate buffer is more stable than PME in crude extract, capsicum puree, and pieces, in that order (Castro et al., 2005). This was explained to be due to differences in the amount of the pressure labile PME fraction in the different systems. Van Buggenhout et al. (2006b) reported that treatment of carrot POD in a cell-free extract at pressure ranging from 100 MPa to 500 MPa did not have any effect on the activity of the enzyme while 50% inactivation was observed in carrot pieces treated at 100–200 MPa for 13 minutes. Similarly, Indrawati et al. (2000, 2001) reported that green beans LOX showed higher sensitivity towards pressure in intact green beans compared to green beans juice. Numerous publications have reported higher rate of inactivation of orange PME in orange juice (Nienaber and Shellhammer, 2001a; Polydera et al., 2004) compared to that reported for the inactivation of the enzyme in model systems (Van den Broeck et al., 2000a), which may be due to varietal differences or based on which of the multiple forms of PME (Versteeg et al., 1980) in orange were dominant in the different studies.

Protective effects of some food components and additives against inactivation have been confirmed in many studies in model systems. Van den Broeck et al. (1999) observed decreased sensitivity of orange peel PME towards high-pressure inactivation at higher soluble solid contents obtained by adding sucrose or calcium chloride into the model solution. For instance, the rate of inactivation in the presence of 20% sucrose was less than half of that without sucrose. Increasing the soluble solid content (Brix) led to similar decrease in the rate of inactivation of PME in orange juice, with *D* values of 91 and 750 minutes at 300 MPa and ambient temperature for 10 and 40 °Brix juices, respectively (Basak and Ramaswamy, 1996). Similar results were reported for POD in guava juice (Lin and Yen, 1998). Likewise, Baron et al. (2006) reported that the presence of sodium pectate improved the pressure stability of purified apple PME with 10% to 30% less inactivation at 100–650 MPa. The inactivation rate of enzymes has also been reported to be dependent on enzyme concentration. The sensitivity of orange peel PME towards pressure inactivation decreased with increased enzyme concentration, especially under conditions of higher rates of inactivation (Van den Broeck et al., 1999). On the other hand, some of the commonly used antibrowning agents such as ascorbic acid, cystein, benzoic acid, gluthathione, citric acid, and sodium chloride have been shown to act in synergy with high pressure on PPO inactivation

from various sources (Weemaes et al., 1997a; Guerrero-Beltran et al., 2005a). When investigating the high-pressure stability of PME in cloudy apple juice, Baron et al. (2006) observed a positive correlation between residual PME activity and the residual content of catechins in the juice indicating that polyphenoloxidase and oxidized polyphenols play a role in the PME activity level after high-pressure treatment. The results suggested that oxidized catechins have PME inhibitory activity so that when PPO is activated by HPP in situ, PME was inhibited (Baron et al., 2006).

Medium pH. The pH of the medium significantly affects the stability of enzymes during processing. The susceptibility of many enzymes towards inactivation increases with decrease in pH. Basak and Ramaswamy (1996) reported that the inactivation rate of PME in orange juice was dependent on the pH. The authors observed higher inactivation rate of PME in orange juice at pH 3.2 compared to the natural pH of the juice (pH 3.7). Similarly, Van den Broeck et al. (1999) observed higher rate of pressure inactivation of the thermolabile orange PME in buffer of pH 3.7 compared to deionized water at a higher pH (4.5) with significant inactivation occurring at pressures as low as 400 MPa at 25°C in the buffer compared to 750 MPa in deionized water (Van den Broeck et al., 1999). Significant differences were observed in the susceptibility of PME to pressure inactivation in Valencia and Navel orange juice; 45% inactivation of PME was observed in Navel orange juice (pH 3.7) compared to no inactivation in Valencia orange juice (pH 4.3) after one-minute treatment at 600 MPa and 20°C. Further investigation showed that this was due to the differences in the pH of the juices. Reducing the pH of Valencia orange juice by adding concentrated HCl resulted in a similar level of PME inactivation as that of Navel orange juice (Bull et al., 2004). Crelier et al. (2001) observed a significant effect of pH on the stability of tomato PME towards higher pressure with the enzyme being less stable at pH 4.2 compared to pH 7.0 in buffer (Crelier et al., 2001). Similarly, D values of 11.5, 33.5, and 64.8 were determined for the inactivation of purified heat labile carrot PME at 750 MPa and 25°C at pH 4.5, 5.5, and 6.0, respectively, indicating significantly higher-pressure stability at higher pH (Balogh et al., 2004). Likewise PPOs from various sources have been reported to be more susceptible to pressure inactivation at lower pH (see section "PPO" under heading "The Effects of High Pressure on Enzyme").

Nevertheless, not all enzymes are sensitive to inactivation at low pH. For instance, tomato PG was rendered more pressure sensitive in buffer at pH 7.0 compared to pH 4.2 both in buffer and tomato juice. The rate of inactivation of the enzyme was much higher in the buffer at pH 7 compared to pH 4.2, both in buffer and juice at 300 MPa and 45°C. According to the authors, increasing the pH may have favored the dissociation of PG1 and increased the proportion of PG2 thereby enhancing the sensitivity of the enzyme towards pressure inactivation (Crelier et al., 2001). Earlier studies showed that PG1 dissociates to PG2 and the β subunit at pH 11 in 0.2 M glycine buffer (Pressey, 1986). Unlike PPOs from other sources, high-pressure treatment of crude apple PPO at 100–300 MPa led to significant increase in activity at lower pH (pH 4.5 and 5.5), whereas inactivation was observed under the same condition at pH 7.0. Complete inactivation of the enzyme occurred after one-minute treatment at 500 MPa at pH 7.0 while the same activity as the untreated sample was observed after one-minute treatment at 500 MPa at pH 4.5 and 5.5 (Anese et al., 1995). In the case of guava and carrot POD, lowering the pH resulted in increased inactivation while increasing the pH increased the inactivation of POD studied by Seyderhelm et al. (1996). Obviously, the effect of pH on the stability of enzymes depends on their structure and biochemical properties. Different effects may also be observed at the same pH, when using different buffers under pressure. For instance, the inactivation rate constant of LOX after CO_2 flushing (H_2CO_3, pH 6.8) and treatment at 400 MPa was 2–10 times higher than the rate of inactivation in McIlvaine buffer of almost the same pH (pH 6.6). Higher pressure stability was also observed in Tris-HCl buffer (pH 9.0) compared to McIlvaine buffer of the same pH. These observations were attributed to the difference in dissociation volumes between Tris-HCl buffer, McIlvaine buffer, and H_2CO_3; the latter two have high dissociation volumes and a larger pH change is expected under pressure in those buffers (Ludikhuyze et al., 1998c).

Temperature. Temperature has a significant impact on the stability of enzymes towards HPP. A lowering of the threshold pressure of inactivation both at high temperature and subambient temperature (cold denaturation) is commonly observed (Hendrickx et al., 1998). Above ambient temperature, an increase in temperature at constant pressure typically leads to an increase in the rate of inactivation of enzymes. From the foregoing discussion, it is clear that in the high-pressure range that is commonly used in food processing ($\geq$500 MPa), pressure and temperature have synergistic inactivation effects. Thus, mild temperature can be used as a hurdle in HPP of foods for more effective inactivation of quality-degrading enzymes. Lower temperature has also been shown to increase the sensitivity of some enzymes. Among plant enzymes, LOXs have been shown to be particularly sensitive to pressure inactivation at subambient temperature conditions (see section "LOX" under heading "The Effects of High Pressure on Enzyme"). Some sensitization of tomato PG2 to pressure inactivation has also been observed at relatively low pressure and subzero temperature conditions. HPP of purified tomato PG2 at –20°C for 10 minutes resulted in 76.6% and 70% inactivation at 100 MPa and 200 Mpa, respectively (Van Buggenhout et al., 2006b).

On the other hand, low temperature with or without freezing does not substantially sensitize PME, PPO, and POD to pressure inactivation (Indrawati et al., 1998; Van Buggenhout et al., 2006b). No significant inactivation of orange peel PME was observed after 10-minute treatment at temperatures as low as –20°C and pressures up to 500 MPa. Similarly, no significant inactivation of tomato PME in cell-free extract and tomato pieces was observed after 30-minute treatment at temperatures between 20°C and –26°C and pressure between 0.1 MPa and 500 MPa. Low temperature had a limited effect, both on

purified horseradish POD and carrot POD in cell-free extract and carrot pieces. Combined high pressure-low temperature treatment of up to 300 MPa and temperatures down to 0°C did not have any effect on purified horseradish POD in phosphate buffer (pH 7.0). Some inactivation of horseradish POD was observed when the temperature was decreased to –20°C, which increased with the treatment pressure. The maximum inactivation was 41.1% at 300 MPa and –20°C after 10-minute treatment. With respect to carrot POD, high pressure of up to 500 MPa at ambient condition did not have any effect on the activity of POD in the cell-free extract, while 30% inactivation of POD was observed when the treatment was carried out at –20°C for 13 minutes. In the case of carrot pieces, high pressure of 100 MPa to 200 MPa in combination with –10°C caused about 50% inactivation of POD after 13-minute treatment time, which is equivalent to what is observed at ambient temperature condition. Likewise, high pressure-low temperature treatment had a limited effect on PPO. No significant inactivation of potato PPO was observed after 13-minute treatments at pressures of 100 MPa to 500 MPa and temperature of –26°C to 20°C, both in cell-free extracts and potato pieces, while a maximum of 36% inactivation occurred at 300 MPa and –20°C after 10-minute treatment of purified mushroom PPO in buffer (Van Buggenhout et al., 2006b). Pressure shift freezing had limited effects on broccoli PPO and POD (Prestamo et al., 2004), horseradish POD, mushroom PPO, and orange peel PME (Indrawati et al., 1998).

Continuous versus cyclic pressure application. Some studies have compared the effects of continuous versus cyclic application of pressure on enzyme inactivation. The results vary depending on the enzyme and its source. For instance, cyclic application of pressure of up to 800 MPa did not have significant effect on tomato PME (Crelier et al., 1995). On the other hand, oscillatory HPP enhanced the inactivation of LOXs. Palou et al. (2000) studied the effect of pressure at 689 MPa with holding times of 5, 10, 15 and 20 minutes in a continuous mode as well as cyclic application with two, three, and four cycles of five minutes each at 689 MPa and two cycles of 10 minutes each at the same pressure. Continuous treatment for five minutes resulted in about 59% inactivation of LOX while 15-minute treatment caused complete inactivation. After 10-minute continuous treatment, 95% inactivation of the enzyme was observed while two cycles of treatment of five minutes each caused complete inactivation of LOX (Palou et al., 2000). Similarly, Ludikhuyze et al. (1998a) observed increased inactivation of soybean LOX in Tris-HCl buffer (pH 9.0) with increase in the number of cycles for the same total treatment time. This effect was found to be more pronounced at low temperature. For instance, 15-minute continuous treatment at 500 MPa and 10°C resulted in 57% inactivation of LOX while three five-minute cycles at the same condition resulted in 90% inactivation of the enzyme (Ludikhuyze et al., 1998a).

Some effects of cyclic pressure application have been reported on avocado PPO. Palou et al. (2000) compared the effect of continuous and oscillatory HPP on the activity of PPO in guacamole (pH 4.3). A continuous treatment of five minutes at 689 MPa caused 49% inactivation, while 20-minute treatment caused 78% inactivation. Cyclic application of pressure somewhat improved the level of inactivation. The maximum level of inactivation was obtained with cyclic treatment with four five-minute cycles causing 86% inactivation. Two cycles of treatment with 10-minute duration had slightly less effect with 80% inactivation (Palou et al., 2000). Cyclic treatment did not have significant effect on the activity of PPO in guava juice compared to a continuous treatment at the same pressure for the same total treatment time (Lin and Yen, 1998). In general, cyclic application of pressure has been more effective for the inactivation of LOX compared to other enzymes such as PME and PPO, possibly due to the sensitivity of the former to low temperature. During cyclic application, depressurization results in a decreased temperature, which affects the temperature reached at the next phase of pressurization. Thus, the gradual decrease in temperature seems to be responsible for the observed effect (Ludikhuyze et al., 1998a). Nevertheless, this is unlikely to be repeated in industrial-scale equipment since heat transfer to the center of the vessel is significantly slowed due to bulk effects.

Activation Versus Apparent Activation

Increased activity of plant enzymes following high-pressure treatment have been reported in many instances. However, a distinction needs to be made between true activation of enzymes through conformational changes and "apparent activation" in tissue systems due to increased release of the enzyme after processing. Increase in the activity of enzymes as a result of HPP may occur due to pressure-induced modification of the secondary and tertiary structure of the enzyme. However, this can also be caused by increased release of membrane-bound enzymes due to substantial tissue and membrane disruption in tissue systems brought about by high-pressure treatment.

In most of the reported activation of enzymes after HPP of tissue systems, increased release of membrane-bound enzymes is probably the predominant cause of the observed apparent activation. Treatment of cloudy apple juice at pressures up to 650 MPa at 25°C led to an increase of PME activity (Baron et al., 2006). A similar increase in PME activity was observed after high-pressure treatment of capsicum puree and pieces at 25°C and 40°C at pressures between 100 MPa and 300 MPa. Such increases in activity have also been reported for PPOs from apple (Bayindirli et al., 2006; Buckow et al., 2009), mushroom (Matser et al., 2000), banana (Palou et al., 1999), tomato (Plaza et al., 2003), strawberry (Terefe et al., 2010), and raspberry (Garcia-Palazon et al., 2004); PODs from apple (Prestamo et al., 2001), tomato (Plaza et al., 2003), strawberry (Garcia-Palazon et al., 2004), and orange (Cano et al., 1997); and LOXs from green peas (Indrawati et al., 2001) and tomato (Rodrigo et al., 2007), among others. This may also contribute to the observed apparently low or no inactivation of some enzymes in tissue systems compared to inactivation of their purified form in model systems; i.e., apparent activation due to extraction may counteract the inactivation effect of pressure in tissue system.

On the other hand, the reported increase in activity of crude and purified enzyme extracts after pressurization can possibly be attributed to conformational changes in the structure of the enzyme. Changes in interaction with other constituents in the extract under pressure may also contribute to the observed activation (Butz et al., 1994). Such activations have been mostly reported for PPOs (Asaka and Hayashi, 1991; Butz et al., 1994; Anese et al., 1995; Gomes and Ledward, 1996; Terefe et al., 2009b), and PODs (Anese et al., 1995; Terefe et al., 2009c) to a lesser extent. With respect to PPO, activation of latent forms of the enzyme by pressure may also occur. Latent forms of PPO have been reported in many plants including carrots (Soderhall, 1995), apples (Yemenicioglu et al., 1997), grapes (cv. Napoleon and cv. Dominga; Nunez-Delicado et al., 2005; Nunez-Delicado et al., 2007), and peach (cv. Catherina; Cabanes et al., 2007). These latent PPOs are activated by treatments such as exposure to detergents (SDS), acid pH, unsaturated fatty acids, proteases (Lei et al., 2004), and heat (Soderhall, 1995; Yemenicioglu et al., 1997). According to Lei et al. (2004), these different treatments activate latent PPO by the same mechanism, i.e., through conformational changes that wipe off the extension polypeptide that shield the active site of the enzyme in the latent PPO. Pressure may also cause the activation of latent PPO through a similar mechanism. This has been confirmed on the latent PPO from "La France" pear. Treatment of the purified latent PPO by high pressure between 400 MPa–700 MPa and 20°C resulted in significant activation of the enzyme with the activity increasing with treatment time (up to six hours), which was assumed to be due to limited conformational changes by pressure. The highest activation occurred at 600 MPa (Asaka et al., 1994).

Catalytic Activity of Enzymes Under Pressure

High pressure may affect the catalytic activity of enzymes in different ways. In principle, high pressure favors reactions accompanied by a volume reduction. However, it may also cause the denaturation of the enzyme that may counteract the pressure induced-acceleration of the enzymatic reaction. Pressure may also cause conformational changes in the substrate as reported for cellulases (Murao et al., 1992) and proteases (Stapelfeldt et al., 1996), which may favor or inhibit the reaction. Thus, the observed overall effect would be the combination of the effects of pressure on the enzyme, the substrate, and the reaction catalyzed by the enzyme. One of the plant enzymes that have been extensively studied in this respect is PME. To some extent, PG, β-glucosidase, and myrosinase have also been investigated.

PME. Several studies have shown that high pressure enhances the PME-catalyzed de-esterification of pectin. It has been proposed that the solvation of charged groups is accompanied by volume reduction resulting from electrostriction, i.e., the compact alignment of water dipoles owing to the columbic field of the charged groups (Balny and Masson, 1993; Mozhaev et al., 1994). PME releases de-esterified (charged) galacturonate residues from pectin, and thus reducing the total volume of the reaction partners. Since any reaction accompanied by a volume

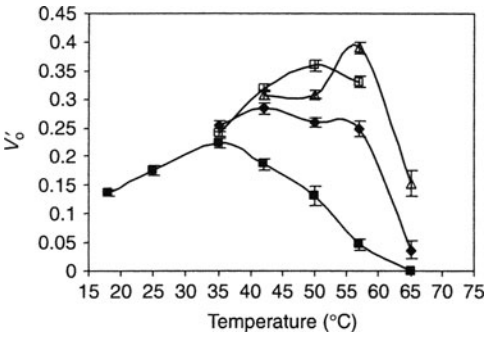

Figure 3 Normalized tomato PME activity versus temperature at atmospheric pressure (■), 150 MPa (♦), 300 MPa (□), and 450 MPa (△) at pH 4.4. (Verlent et al., 2004a). Reproduced with permission of Wiley & Sons.

reduction is favored under pressure, it is not surprising that the catalytic activity of PME is significantly accelerated under pressure. The increase in activity is not only dependent on pressure and temperature, but also on environmental factors such as pH and presence of sugars, salts, or other food additives (Verlent et al., 2004a).

Tomato PME has been found to have a three- to four-fold higher activity on pectin at elevated pressure than at atmospheric pressure (Van den Broeck et al., 2000b; Verlent et al., 2004a; Verlent et al., 2007). Pressure shifts the temperature of optimal enzyme activity towards higher temperatures (see Fig. 3). Tomato PME activity under pressure is highest at pH 8.0, whereas acidification significantly decreases the demethoxylation of pectin (Verlent et al., 2004a). Tomato PME activity is also influenced by the presence of active tomato PG. Verlent et al. (2007) observed a lower tomato PME activity in the absence of PG than in presence of PG at atmospheric pressure. The PME-catalyzed pectin de-esterification is accelerated by increasing pressure up to 200 MPa in the presence of tomato PG. Higher pressures diminished PME activity to values even lower than atmospheric pressure, which is in contrast to what has been reported with respect to PME activity in the absence of tomato PG (Verlent et al., 2007).

Pressure also proves to be very efficient in stimulating the catalytic activity of PME from capsicum (Castro et al., 2006a). The purified enzyme showed a maximum activity at 200 MPa and 55°C with approximately 20% higher activity compared to optimal conditions at ambient pressure (∼50°C). PME activity in shredded carrots was found to be approximately doubled when increasing the pressure from atmospheric to 300–400 MPa at constant temperature (Sila et al., 2007). Duvetter et al. (2006) reported a highly accelerated pectin conversion by fungal PME under elevated pressure/temperature conditions. However, no significant effect of increasing pressure up to 300 MPa and/or temperature on the mode of pectin conversion was detected. The mode of pectin demethoxylation remained random (Duvetter et al., 2006).

PG. Verlent et al. (2004b, 2005) investigated the effect of high pressure and temperatures on the catalytic activity of PG purified from tomatoes (40 mM or 100 mM Na acetate buffer,

pH 4.4). At atmospheric pressure, the highest enzymatic conversion of polygalacturonic acid was found at 55–60°C. When the pressure was increased up to 200 MPa, the enzyme showed a significant deceleration of its catalytic activity and this decrease was more pronounced at higher temperatures. For instance, at 100 MPa and 50°C, the catalytic rate was just 30% of the rate at 50°C and ambient pressure conditions (Verlent et al., 2004b, 2005).

In a subsequent study, the catalytic activity of purified tomato PG under combined pressure–temperature conditions was investigated in the presence and/or absence of tomato PME (Verlent et al., 2007). In general, tomato PG activity was influenced indirectly by the presence of active tomato pectinmethylesterase and vice versa during thermal and high-pressure treatment. Noticeable depolymerization of highly esterified pectin by tomato PG was observed when active tomato PME was present, whereas almost no activity was found in absence of tomato PME. In the pressure range 100–200 MPa, an increase in PG activity was observed in the presence of PME due to an increased accessibility of de-esterified pectin. At higher pressures, the rate of enzymatic pectin depolymerization diminished probably because of PG inactivation. The optimal condition to preserve or improve rheological properties of tomato-based products were found at 40°C and 400 MPa, because at this condition tomato PG activity is reduced while PME activity is maintained.

Pressure also affected the action pattern of tomato PG on de-esterified pectin by tomato PME or by *A. aculeatus* PME (Verlent et al., 2005). Although both pectic substrates had the same degree of esterification, the initial PG activity and its action pattern differed because of the difference in distribution of methyl ester groups along the pectin chains after the action of tomato PME (blockwise) or fungal PME (random). Randomly de-esterified pectins were found to be less sensitive to breakdown by tomato PG than blockwise de-esterified pectins during thermal and/or pressure treatment. Thus, fungal PME can be used to protect against and/or control changes of texture and rheology of fruit- and vegetable-based products caused by PG activity. Compared to ambient pressure conditions, partially purified cabbage PG showed a drastic decrease of its catalytic activity after one-minute treatment at 400 MPa resulting in an increase of the Michaelis Menten constant K_m and a decrease of the maximal reaction rate V_{max}. Pressure treatment also caused a slight shift of the pH and temperature optimum for PG activity (Choi et al., 1999).

Potential implications of the high pressure-induced changes in the activity of pectinases. The enhanced activity of PME under pressure coupled with the sensitivity of PG towards pressure inactivation opens the possibility for tailored modification of plant polysaccharides that can be applied for reducing textural loss or modulating texture in processed horticultural products (Van Buggenhout et al., 2009). A production process for fruit gel snacks, which is based on the activation of PME under pressure, is already patented by Mars (Jacops et al., 2007). There are also a number of studies on the application of PME and/or calcium infusion together with high pressure pre-treatment for improving the textural quality of processed fruit and vegetable products. Duvetter et al. (2005) observed that vacuum-assisted (10 hPa) infusion of strawberry halves with PME (100 U/mL) and calcium chloride (0.5% w/w) prior to thermal (60–80°C) and high pressure (400–550 MPa) treatment significantly improved the texture retention of the processed strawberries. The highest increase in firmness was correlated with the highest decrease in the degree of methylation of pectin, showing that the increase in firmness is mainly due to the action of the infused PME. The high pressure-treated vacuum-infused samples showed higher retention of texture regardless of treatment pressure and time while treatment temperature had a significant effect on the thermally treated vacuum-infused samples. Vacuum-infused samples treated at 60°C for up to 20 minutes had as high firmness values as fresh samples while much higher firmness was observed in the high pressure-treated ones. Regardless of the pressure and treatment time, vacuum-infused samples treated by high pressure were about twice as firm as the fresh samples. Without vacuum infusion pre-treatment, the strawberries lost 80% of their firmness after HPP. Both tomato and fungal PME were used in the experiments; however, fungal PME had the greatest effect on texture perhaps due to its random mode of pectin de-esterification as opposed to the blockwise de-esterification of pectin by tomato PME (Duvetter et al., 2005). Likewise, vacuum infusion of fungal PME (100 U/mL) and calcium (0.5%) combined with rapid cryogenic or high pressure shift freezing significantly improved the texture of frozen strawberries. Vacuum-infused strawberry halves showed 58% retention of their firmness after cryogenic freezing and thawing compared to 12% in untreated samples. Vacuum-infused samples frozen by high pressure shift freezing were firmer than the fresh samples (Van Buggenhout et al., 2006a).

Sila et al. (2004, 2006) studied the effect of high pressure pre-treatment on the textural degradation kinetics of carrot during thermal processing at 90–110°C. High pressure pre-treatment of carrots for 15 minutes at pressure ranging from 200 MPa to 400 MPa caused up to a four-fold decrease in the rate of textural degradation during thermal processing. The highest effect was observed at 60°C and 400 MPa. High pressure pre-treatment resulted in a larger decrease in the degree of methylation of pectin as well as lower texture degradation rate compared to low-temperature blanching (60°C, 40 minutes), which was attributed to tissue disruption by high pressure allowing contact between the enzyme and the substrate and increased PME activity under pressure. The reduced rate of textural degradation is mainly due to the reduced rate of β-elimination as the reaction favors highly methylated pectins. The decreased texture degradation kinetics was correlated with the reduction in the degree of methylation of pectin in carrot tissue. Calcium soaking increased the residual hardness from 10% to 20% and further decreased the rate of textural degradation (Sila et al., 2004, 2006). Similarly, Van Buggenhout et al. (2005) observed improved texture of frozen carrots by combining pre-treatment at high pressure (300 MPa, 60°C, 15 minutes) or mild temperature (30 minutes, 60°C) with high pressure shift freezing. This was accompanied

by a reduction in the degree of methylation of pectin. In the untreated samples, high pressure shift freezing did not have significant advantage compared to conventional freezing in terms of texture. Pre-treatment also did not have significant effect on the texture of conventionally frozen carrots (Van Buggenhout et al., 2005). HPP of bilberries and black currants resulted in a significant decrease of the degree of methylation of pectin, most probably through the activation of endogenous PME. Combining high pressure with commercial pecteolytic enzyme preparations resulted in a higher degradation of the cell wall polysaccharides of the berries (Hilz et al., 2006).

β-glucosidase. The effects of temperature and pressure on the activity of almond β-glucosidase were investigated with p-nitrophenyl-β-D-glucopyranose as a substrate. Increasing pressure did not have any effect on the optimal temperature for the reaction, which was 60°C both at atmospheric pressure and at 150 MPa. At 40°C and 60°C, increased pressure up to 250 MPa resulted in decreased rate of reaction. At all pressure conditions, the reactions followed Michaelis-Menten kinetics. The V_{max} decreased with increasing pressure while K_m remained constant with a consequent decrease in the catalytic efficiency (k_{cat}/K_m) of the enzyme. The activation volume of the overall reaction at 60°C was positive (22.1 mL/mol) and constant up to 250 MPa (Hamon et al., 1996).

Myrosinase. Moderately high pressure has been shown to enhance the catalytic activity of myrosinases from broccoli and mustard seed. The optimal temperature for the activity of crude broccoli myrosinase in buffer (pH 6.55) was 30°C at ambient pressure, which remained the same at elevated pressure. Increase in pressure to 50 MPa slightly enhanced the activity of crude broccoli myrosinase at 20–50°C. Further increase in pressure to 200 MPa led to decreased activity due to the pressure inactivation of the enzyme. The maximum activity was observed at 50 MPa and 30°C, which was about 8% higher than the activity at ambient pressure (Ludikhuyze et al., 2000). The activity of broccoli myrosinase under pressure was also investigated in broccoli juice from which the endogenous glucosinolates were removed and singrin was added as an artificial substrate. The optimal temperature for the activity of the enzyme was 40°C at ambient pressure, which shifted to 45°C at elevated pressure. The activity of myrosinase increased when pressure was increased from 50 MPa to 100 MPa at temperatures between 20°C and 50°C. Nevertheless, the maximum activity was observed at atmospheric pressure (Van Eylen et al., 2008b). Study in intact broccoli heads showed that application of high pressure promotes the enzymatic conversion of broccoli glucosinolates into the bioactive isothiocyanates even at pressures as high as 500 MPa at ambient temperature, which is sufficient for blanching. This is due to high pressure-induced cell disintegration and enzyme–substrate decompartmentalization and since processing at ambient to mild temperature conditions helps to maintain the stability of glucosinolates and their hydrolysis products as opposed to thermal blanching (Van Eylen et al., 2008b, 2009).

The activity of mustard myrosinase was studied in model system with singrin as a substrate (Bis-Tris buffer, pH 6.5) with or without ascorbic acid and $MgCl_2$ and in broccoli juice (in which the endogenous myrosinase was thermally inactivated) at 0.1–600 MPa and 10–70°C. Maximum activity at ambient pressure in the model system was observed in the presence of 0.15 mM $MgCl_2$ and 3.38 mM ascorbic acid. The optimum temperature at ambient pressure was 60°C, which shifted to 40°C at elevated pressure. Increase in pressure up to 300 MPa enhanced the activity of the enzyme at all temperatures investigated. The maximum activity was observed at 200 MPa and 40°C, which was almost twice the optimum activity at atmospheric pressure. The activity decreased at pressures higher than 200 MPa with no activity detected at 600 MPa. The optimum temperature for the activity of the enzyme in broccoli juice at ambient pressure was 60°C. Unlike in the model system, it remained the same at elevated pressure. Higher activity was observed at elevated pressure up to 400 MPa compared to ambient pressure (Van Eylen et al., 2008a). The maximum activity was observed at 200 MPa and 60°C and was about eight times higher than the optimal activity at atmospheric pressure. Clearly, mustard myrosinase can potentially be used as an adjuvant in the HPP of cruciferous vegetables for modulated production of isothiocyanates.

CONCLUSIONS

High-pressure processing is a useful alternative to thermal processing for the preservation and processing of food products. HPP at 600 MPa inactivates vegetative microbial cells and at least partially inactivate many quality-degrading enzymes at around room temperature, resulting in a very high retention of the nutritional and sensory characteristics of the fresh product. Unfortunately, HPP under commercially feasible pressure conditions can not inactivate most bacterial endospores at ambient to mild temperature conditions, which limit its application mainly to the pasteurization of acid products, cooked vegetables, and meat destined for distribution through the cold chain. However, pressure-assisted thermal sterilization is now an approved technology for sterilization of some low acid foods such as mashed potato. The effect of this process on the sensorial and nutritional quality of foods remains to be fully investigated and reported.

In general, enzymes are more resistant to inactivation than vegetative microorganisms and thermal processing is usually the most cost-effective way of enzyme inactivation. Enzymes such as PPO and PME are highly resistant to high pressure, although their sensitivity depends on their origin as well as their environment. Thus, at economically feasible high-pressure conditions, at most they can only be partially inactivated. The exceptions are PG and LOX, which are relatively more pressure-sensitive plant enzymes studied so far and can be substantially inactivated by high pressure at commercially feasible conditions. Nevertheless, HPP at room to mild temperature is the best alternative to thermal preservation, if maintaining fresh-like sensory and nutritional quality are the main considerations. Moreover, the retention of enzymes may add to the natural and "fresh like"

characteristics of high pressure-processed products, which may make the process attractive for organic and all-natural certification. The retention and activation of enzymes such as PME by high pressure is also useful for improving the texture of processed fruit and vegetable products as well as for creating novel structures that are not feasible with thermal processing. Thus, the technology has the potential to be used in the development of a whole new generation of value-added foods possibly with unique/or novel functional properties.

Obviously, enzyme mediated quality degradations can occur during storage of high pressure processed products. Often, these may be controlled to a large degree through the use of appropriate packaging, oxygen scavengers, and/or enzyme inhibitors as well as refrigerated storage. Mild heat is another way of enhancing the efficiency of high pressure for enzyme inactivation. There are several studies that indicate that better degree of microbial and enzyme inactivation can be achieved by treatments at lower pressure (300–500 MPa) in combination with mild heat (40–70°C) compared to HPP at room temperature condition. Such processes have also been shown to result in high physical and nutritional quality retention, especially in puree and juice products where texture is not the most important quality parameter. Nevertheless, considering the additional capital and operating costs associated with HPP, its application in the foreseeable future will be limited to high value "niche" products where there are significant quality or shelf life extension benefits and return on capital expenditure can be assured. In view of this fact, thermal processing will continue to be the major food preservation technique of commodity products in the coming years.

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