

---

# 25 Enhancing the Nutritional Quality of Fruit Juices

## *Advanced Technologies for Juice Extraction and Pasteurization*

*Robert D. Hancock and Derek Stewart*

### CONTENTS

25.1 Introduction .....	463
25.2 Optimization of Fruit Extraction for Enhancing The Nutritional Content of Juice Products .....	464
25.3 Novel Technologies for the Pasteurization and Preservation of Fruit Juices: Impact on Nutritional Quality .....	467
25.3.1 Pulsed Electric Fields .....	468
25.3.2 High Hydrostatic Pressures .....	470
25.3.3 Dense Phase CO <sub>2</sub> .....	473
25.3.4 Ultrasound .....	474
25.3.5 Ultraviolet Light .....	475
25.3.6 Ionizing Radiation .....	476
25.4 Conclusions .....	476
References .....	477

### 25.1 INTRODUCTION

For many years epidemiological studies have highlighted a strong negative correlation between fruit and vegetable intake and incidence of degenerative diseases such as numerous forms of cancer (Block et al., 1992), cardiovascular diseases (Ness and Powles, 1997), and other diseases including age-related macular degeneration (Goldberg et al., 1988), cataract (Mares-Perlman et al., 1995), bronchitis, asthma, peptic ulcers, gallstones, liver cirrhosis, kidney stones, and arthritis (La Vecchia et al., 1998). One suggestion for the protective effects of diets high in fruits and vegetables is their high content of antioxidant phytochemicals, which are proposed to protect vital biomolecules such as DNA, proteins, and lipids from oxidation caused as a result of the production of free radicals under both normal metabolism and conditions of stress. Over time and with insufficient repair, the damage caused by molecular oxidation is proposed to result in disease (Diplock et al., 2003).

As a result of the epidemiological evidence, many governments have introduced campaigns to educate citizens with regard to the importance of fruit and vegetable consumption (e.g., the United Kingdom 5-a-day campaign), and a recent World Health Organization expert consultation suggested the consumption of at least 400 g of fruits and vegetables per day to minimize the risk of chronic disease (WHO/FAO, 2003). Fruit juices can make an important contribution to fruit and vegetable consumption, with fruit juice comprising approximately 18% of the total intake of the adult UK

population (Henderson et al., 2002). Furthermore, several intervention studies have shown improved antioxidant status following fruit juice consumption (Netzel et al., 2002; Bub et al., 2003; García-Alonso et al., 2006; McGhie et al., 2007). However, in recent years there has been increased skepticism regarding the antioxidant hypothesis, with several intervention trials suggesting that antioxidant supplementation may have adverse effects (Selman et al., 2006; Ristow et al., 2009) and a recent systematic review suggesting that antioxidant supplementation may increase all-cause mortality (Bjelakovic et al., 2007). As a result of these findings, researchers are now beginning to focus on mechanisms of protection unrelated to the *in vitro* antioxidant capacity of phytochemicals (Hancock et al., 2007; Stevenson and Hurst, 2007).

Whatever the merits of the antioxidant hypothesis, consumer opinion is firmly in favor of antioxidant consumption and many juice manufacturers have exploited these beliefs in their marketing campaigns. Furthermore, there is a strong consumer demand for premium and “natural” products with minimum processing and no additives. As a result, a key to improving competitiveness for juice manufacturers will be to provide high-quality, fresh-tasting juices that maintain a nutrient profile as close as possible to that of fresh fruits. In the current chapter we discuss modern manufacturing technologies for maximizing the nutritional properties of fruit juices. In particular, the impacts of nonthermal extraction and pasteurization technologies on the antioxidant capacity and content of antioxidant compounds in fruit juices are highlighted. Furthermore, the benefits of such technologies in maintaining the nutritional quality of fruit juices over shelf life are discussed.

## 25.2 OPTIMIZATION OF FRUIT EXTRACTION FOR ENHANCING THE NUTRITIONAL CONTENT OF JUICE PRODUCTS

Historically, fruit juice manufacturers have focused on maximizing juice yield achieved through physical and (bio)chemical treatments designed to weaken plant cell walls and loosen membranes and through process engineering solutions designed to optimize press design and juice recovery. More recently, it has become clear that fruit pretreatments and extraction methods can have significant impacts on the nutritional quality of the product. The present section focuses on recent work aimed at maximizing the extraction of wall-associated polyphenols by optimization of enzymatic maceration treatments.

Hydrolytic enzymes such as pectinases, cellulases, and amylases have been used in the juice processing industry for many years to improve juice yields, improve rheological properties, remove suspended matter in the production of clear juices, and improve cloud stability in cloudy juices (Kashyap et al., 2001). Pectinases are by far the most widely used, accounting for 25% of global sales of food enzymes (Jayani et al., 2005) with an estimated annual market value of \$150 million in 2005 (Kashyap et al., 2001). Acidic pectinases used in fruit juice production are mainly produced by fungi and yeasts in either submerged or solid state culture with *Aspergillus niger*, the most commonly used species for commercial production (Favela-Torres et al., 2006). Commercially available pectinases are not single purified proteins but mixtures of enzymatic activities including polygalacturonase, pectin lyase, pectin methylesterase, endoglucanase,  $\beta$ -glucanase, xylanase, mannanase,  $\alpha$ -arabinosidase,  $\beta$ -arabinosidase,  $\beta$ -galactosidase, and  $\beta$ -glucosidase activities (Table 25.1). Specific enzyme preparations are tailored for and find specific applications. In recent years it has become clear that careful selection of the appropriate pectinase preparation is required not only to maximize juice yield and obtain desirable physical properties but also to maximize both initial yield and subsequent stability of health promoting phytochemicals.

Several parameters must be considered for the optimization of enzymatic maceration treatments to maximize the recovery of nutritionally active components into fruit juices. Firstly, the physical association of nutritionally active compounds with insoluble fruit fractions can have a negative impact on recoveries. For example, polyphenolic antioxidants are frequently associated with macromolecular components found in the cell wall and are subsequently lost in fruit pomace. Secondly,

**TABLE 25.1**  
**Relative Glycosidase Activities of Commercial Pectinase Preparations**

Product	Glycosidase Activity										
	PG	PL	PME	EG	$\beta$ -Glu	Xyl	Man	$\alpha$ -Ara	$\beta$ -Ara	$\beta$ -Gal	$\beta$ -Glc
E CE	100	0	0	1300	4700	2300	160	50	0	5	5
B CCM	100	0	14	4	100	48	8	5	0.2	2	0.4
B 8X	100	NA	16	5	NA	25	5	7	NA	2	0
Roh	100	NA	0	10	NA	135	14	1	NA	4	2
P S	100	NA	22	6	NA	2	89	2	NA	5	0
P S XXL	100	890	0	38	98	39	0	0	0	0	0
P BE 3L	100	0	17	8	180	180	11	25	2	23	3
P BEX	100	NA	17	8	NA	72	10	26	NA	12	0
P SP-L	100	NA	9	6	NA	3	55	2	NA	5	0
P 3XL	100	NA	17	5	NA	7	5	27	NA	11	0

*Sources:* All data were obtained from Buchert et al. (2005), Koponen et al. (2008), and Puupponen-Pimiä et al. (2008). Pectinase preparations were E CE, Econase CE; B CCM, Biopectinase CCM; B 8X, Biopectinase Super 8X; Roh, Rohapect; P S, Pectinex Smash; P S XXL, Pectinex Smash XXL; P BE 3L, Pectinex BE 3-L; P BEX, Pectinex BE XXL; P SP-L, Pectinex Ultra SP-L; and P 3XL, Pectinex 3 XL. Enzyme activities were PG, polygalacturonase; PL, pectin lyase; PME, pectin methylsterase; EG, endoglucanase;  $\beta$ -Glu,  $\beta$ -glucanase; Xyl, xylanase; Man, mannanase;  $\alpha$ -Ara,  $\alpha$ -arabinosidase;  $\beta$ -Ara,  $\beta$ -arabinosidase;  $\beta$ -Gal,  $\beta$ -galactosidase; and  $\beta$ -Glc,  $\beta$ -glucosidase. NA, data not available.

conditions of enzymatic maceration are an important consideration with temperature, time, and aeration affecting the recovery of chemically unstable compounds, including many of the antioxidant components. Finally, the specific enzyme activities present in the pectinase preparation selected can have important consequences for both the release of nutritionally important compounds and their subsequent stability.

Pectinase preparations are commonly used in the preparation of berry juices to reduce the viscosity and increase the processibility and juice yields of these high pectin containing fruits (Hilz et al., 2005). Juice yield improvements are typically in the region of 20% (Buchert et al., 2005) and viscosity reduction of expressed juice is up to 80% (Semenova et al., 2006). Enzymatic treatments have been observed to improve total polyphenol (Landbo et al., 2007) and anthocyanin (Koponen et al., 2008) recoveries, and to produce juices with improved antioxidant properties as measured by *in vitro* radical scavenging capacity (Puupponen-Pimiä et al., 2008) and protection of human low-density lipoprotein (LDL) from oxidation (Landbo and Meyer, 2004). The positive impact of pectinase treatment on polyphenol recovery is linked to the improved solubilization of cell walls preventing them from acting as an adherent to which polyphenols can bind, thus partitioning them into the fruit pomace. This mechanism is supported by the observed linear correlation between cell wall degradation and phenolic release in pectinase-treated blackcurrants (Bagger-Jørgensen and Meyer, 2004). Furthermore, berry anthocyanins, which frequently form a large proportion of the total polyphenol pool and impart the vibrant colors desired by consumers, are mainly located in the vacuoles of lysis-resistant epidermal cells, which become significantly more extractable following enzymatic treatments (Ros Barcelo et al., 1994).

At least two studies have systematically examined the impact of varying enzymatic maceration parameters (temperature, time, and enzyme/substrate ratio) on the yield and nutritional attributes of berry juices. When preparing juice from elderberry, maceration time, maceration temperature, and enzyme dose were all positively correlated with juice yields within the range of 7–50 min, 30–62°C, and 0–0.36% enzyme/substrate ratio, respectively (Landbo et al., 2007). However, maceration time had no impact on recovery of anthocyanins or total phenols and only maceration temperature was

correlated with recovery of phenols. Following optimization, ideal maceration conditions were determined as 63°C for 30 min at an enzyme/substrate ratio of 0.12%. The pectinase preparation used for maceration also had an impact, with Pectinex BE Color consistently providing the highest juice yields and phenolic and anthocyanin contents. Similar work undertaken in blackcurrants demonstrated that maceration time (0–60 min), temperature (30–50°C), and enzyme/substrate ratio (0–0.1%) were all positively correlated with phenol recoveries. Of the four enzyme preparations tested, Macer8 [FJ] and Pectinex Ultra SP-L were the most effective in improving both juice yield and phenol concentration; however, the juice produced following Pectinex maceration demonstrated greater inhibition of LDL oxidation *in vitro* (Bagger-Jørgensen and Meyer, 2004). These data emphasize the need to optimize fruit maceration parameters both in terms of physical parameters (temperature, time, and enzyme/substrate ratios) and regarding the specific pectinase preparation used.

Side activities found in many commercial pectinases are capable of hydrolyzing not only cell wall components but also sugar–phenol conjugates generating their aglycone moieties. A number of studies have observed correlations between side activities and the release and subsequent stability of berry juice phenolic components. The importance of judicious selection of the pectinase preparation was highlighted by a comparison of the effects of pectinase treatment on anthocyanin recoveries in bilberry and blackcurrant. Bilberry anthocyanins are highly heterogeneous consisting of glucosides, galactosides, and arabinosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Riihinen et al., 2008), whereas blackcurrant anthocyanins are dominated by four main species, glucosides and rutinosides of delphinidin and cyanidin (McDougall et al., 2005). Treatment of bilberry mash with low doses (1 nkat polygalacturonase activity/g) of Econase CE, Biopectinase CCM, Pectinex Smash XXL, or Pectinex BE 3-L enhanced the recovery of anthocyanin glucosides, arabinosides, and galactosides compared with untreated mash. In addition, increased enzyme dosage up to 100 nkat/g further improved the recovery of glucosides and arabinosides (with the exception of Pectinex BE 3-L), while high doses of all enzymes with the exception of Pectinex Smash XXL reduced the recovery of galactosides (Koponen et al., 2008). Table 25.1 shows the catalytic activities associated with the various enzyme preparations used and suggests that the reduced recovery of galactosides is associated with the  $\beta$ -galactosidase side activity present in all preparations, with the exception of Smash XXL. The nutritional significance of these results relates to the instability of the aglycones, which are subsequently rapidly broken down (Sadilova et al., 2006). On the contrary, the stability of glucosides following Econase CE treatment is more problematic given that the enzyme preparation contains equivalent glucosidase and galactosidase activities. One explanation may arise from the results obtained following the digestion of blackcurrant mash, where no aglycone anthocyanidins were detected and recoveries of both glucosides and rutinosides were enhanced (Buchert et al., 2005; Koponen et al., 2008). These data imply that in blackcurrant, enzymatic digestion improved the release of anthocyanins from the cell wall and rutinosidase and rhamnosidase activities were absent from the pectinase preparations used. The observation that glucosides were spared despite the fact that some preparations contained glucosidase activity suggests that anthocyanidin glucosides are poor substrates for such enzymes or alternatively that blackcurrant extracts contained a glucosidase inhibitor. The latter suggestion was supported by the observation that maceration of blackcurrant with Econase CE, which contains equivalent amounts of galactosidase and glucosidase activity, resulted in a slight reduction of glucosides significantly lower than that observed when the same enzyme was used to macerate bilberries. Further support for the hypothesis that side activities may have negative impacts on anthocyanin stability was obtained following the preparation of juices from strawberry and raspberry where extended incubation periods (2–6 h) resulted in losses of anthocyanins compared with nonenzyme macerated controls. On the contrary, enzymatic maceration resulted in improved or neutral recoveries of quercetins and ellagic acids compared with nonenzymatic maceration (Versari et al., 1997).

One issue affecting a number of juices, and in particular berry fruit juices, is the production of a haze consisting of polyphenol–protein complexes on storage (Siebert, 1999). Numerous fining

agents are used to remove either haze-active proteins (e.g., silica gel) or haze-active polyphenols (e.g., polyvinylpolypyrrolidone); however, the use of such fining agents can result in significant losses in polyphenol and antioxidant content. An alternative to the use of finings is the degradation of proteins by the use of proteases, thus maintaining nutritionally significant polyphenols in solution. In one study, the impact of protease-assisted clarification of blackcurrant juice was compared with conventional clarification using gelatin and silica sol (Landbo et al., 2006). Impacts on haze formation and retention of nutritionally significant polyphenols were examined. Following tests of five different acid fungal protease preparations on immediate turbidity reduction and rate of turbidity increase following storage, Enzeco Fungal Acid Protease was demonstrated to be at least as effective as conventional gelatin–silica treatment in preventing haze formation. However, unlike conventional clarification treatments that removed approximately 30% of the anthocyanins present in juices, protease treatment only decreased juice anthocyanin content by 12%.

As a hard fruit, enzymatic maceration is necessary to improve apple juice yields from below 50% in the absence of enzymes up to 80% in their presence (van der Sluis et al., 2002). Unlike berry fruit, apples contain high endogenous levels of polyphenol oxidase (PPO) and peroxidase, resulting in enzymatic losses of polyphenols through oxidation. This results not only in a reduction in nutritional value but also in reduced organoleptic desirability caused by browning reactions, which can become extensive during extended enzymatic maceration. The impact of PPO was highlighted by the observation that during a 2-hour enzymatic maceration, substantial losses were observed for the PPO substrates phloridzin, chlorogenic acid, and catechin while the nonsubstrates cyanidin galactoside and quercetin glycosides were not affected (van der Sluis et al., 2002). Losses of polyphenolic compounds can be reduced using nonoxidative enzymatic maceration in which ascorbic acid is added, resulting in particularly strong preservation of chlorogenic acid, procyanidins, and epicatechins (Mihalev et al., 2004).

Several authors have examined the impact of using pectinases in conjunction with other hydrolytic enzymes to improve the yields and recovery of nutritionally active biomolecules in apple processing. Frank Will et al. (2000) developed a two-stage process for the production of a premium, cloudy juice following pectinase treatment followed by pomace liquefaction with different pectinases and cellulases for the production of extraction juice. Extraction juices contained between 1.5 and 2.5 times the polyphenol content of premium juices and in addition contained certain polyphenolic substances, including procyanidins and quercetin derivatives, that were absent in the premium juices. Extraction juices also contained approximately 10–20-fold higher colloids (mainly consisting of cell wall-derived oligo- and polysaccharides) than premium juices, acting as a potential source of dietary fiber. Although sensory data were not presented, it would appear that extraction juice could have significant nutritional advantages over premium juices and although pomace liquefaction is currently banned in the European Union it has been used in some North and South American countries.

Enzyme-assisted juice extraction is now widespread in modern juice manufacture, with enzymes often chosen for their yield maximizing potential and impact on juice physical and organoleptic properties. However, it is now becoming clear that the choice of enzymes can also have a significant impact on the nutritional qualities of fruit juices, and manufacturers need to be aware that the choice of enzyme and specific maceration conditions can have both positive and negative impacts.

### 25.3 NOVEL TECHNOLOGIES FOR THE PASTEURIZATION AND PRESERVATION OF FRUIT JUICES: IMPACT ON NUTRITIONAL QUALITY

In 1998 the United States Food and Drug Administration (US FDA) introduced new labeling requirements for fruit juices that had not been treated to achieve a 5 log reduction in pathogen load (Freidman and Shalala, 1998). Taken together with the recognition that traditional thermal pasteurization can have significant negative impacts on the nutritional quality of fruit juices, this ruling has

spurred research into nonthermal methods for the reduction of microbial pathogens while maintaining high nutritional quality. The most promising technologies are discussed.

### 25.3.1 PULSED ELECTRIC FIELDS

Although pulsed electric field (PEF) technology was first introduced in the 1960s (Wouters et al., 2001a), it was not until the development of a continuous treatment chamber in the late 1980s that the use of PEF in food processing became a realistic opportunity (Dunn and Pearlman, 1987).

PEF technology involves the application of short pulses ( $<10 \mu\text{s}$ ) of high voltage ( $20\text{--}80 \text{ kV cm}^{-1}$ ) to fluid foods placed between two electrodes. Pulses can be generated at rates up to 2000 per second and foods are typically subject to a series of pulses where the total time of application is normally less than 1 s (Min et al., 2003a). At present there are a number of commercially available PEF-processed juices and juice blends available in the United States; however, European Union novel food regulations will require the demonstration of substantial equivalence before such products can go on sale in Europe (Min et al., 2007). One particular area of concern is the transfer of metal ions from PEF electrodes into the medium being processed, although under typical processing conditions, such transfer remains below legislation values for fruit juices producing lower contamination than that outlined for water under the European Union Drinking Water Directive (Roodenburg et al., 2005).

Ultrastructural and biophysical data suggest that microbial inactivation occurs as a result of the formation of membrane pores induced by structural fatigue caused by electric field-induced compression and tension. Induction of such pores allows the flow of ions and water, creating further membrane stress that finally leads to cellular swelling or shrinkage and the disruption of cytoskeletal structures (Chang and Reese, 1990). Additional factors affecting eukaryotic microorganisms include organellar disruption and loss of ribosomes (Harrison et al., 1997). Microbial inactivation is dependent on process parameters that can be controlled externally in addition to the intrinsic characteristics of both the microorganism and the product being treated (Table 25.2). For the majority of process parameters, there is a positive correlation with increasing microcidal activity, primarily as a result of increasing energy entering the system. The limiting factors are therefore the time and cost implications of increasing the parameters and the negative impacts of increased energy input on nutritional components, as discussed below. Although limited data are currently available, it appears that square wave pulses in which maximal applied voltage is rapidly achieved and then

**TABLE 25.2**  
**Factors Affecting the Efficacy of Pulsed Electric Field Treatments in Microbial Sanitization**

Process Parameters	Product Characteristics	Microbial Characteristics
Electric field strength (+)	Composition	Cell size (+)
Pulse width (+)	Heterogeneity (–)	Cell morphology
Pulse number (+)	Conductivity (–)	Cellular complexity (+)
Total pulse duration (+)	Ionic strength (–)	Cell type (vegetative/ spore)
Pulse shape	pH	Growth phase
Treatment temperature (+)	Water activity (+)	
	Density (+)	
	Viscosity (–)	

*Note:* Further details are found in the text. +, microcidal effect increases as parameter increases; –, microcidal effect decreases as parameter increases.

removed are more microcidal than pulses in which voltage decays exponentially (Zhang et al., 1994; Pothakamury et al., 1996).

The ionic strength and conductivity of the processed food correlate negatively with the microcidal action of PEF, where low medium electrical conductivity exacerbates the differences between medium and microbial cytoplasm, thereby increasing ionic flow across microbial membranes (Pothakamury et al., 1996). Heterogeneity can lead to ineffective microcidal activities as a result of the formation of microenvironments in which PEF treatment may be less effective and increased viscosity will favor the formation of a heterogeneous fluid. On the contrary, increased water activity is associated with improved microcidal activity of PEF (Min et al., 2002) while higher densities limit temperature changes allowing greater energy input without adversely affecting nutritional qualities. Although pH has been shown to affect microcidal activity using a range of microbes (Vega-Mercado et al., 1996; Liu et al., 1997; Wouters et al., 2001b), the impact of pH was species dependent.

Although different microbial spoilage organisms show very different responses to PEF treatments, a few generalizations can be drawn. Cellular size, morphology, and complexity all affect the microcidal activity of PEF, with smaller, simpler cells being more resistant than larger, more complex eukaryotic cells. For example, bacterial and fungal spores show greater resistance than vegetative bacterial cells, which in turn show greater resistance than yeasts (Grahl and Markl, 1996), while larger cells of the same species show greater susceptibility than smaller cells (Wouters et al., 2001b). In addition, actively dividing cells are more susceptible than those in the stationary phase of growth (Pothakamury et al., 1996; Wouters et al., 1999).

Numerous studies have analyzed the impact of PEF treatments in reducing the microbial load in fruit juices, and these have been previously summarized (Wouters et al., 2001a; Min et al., 2007). Reductions in microbial counts greater than 5 log have been observed in apple, orange, cranberry, grape, and tomato juices. Studies have demonstrated a greater than 5 log reduction against specific microorganisms directly inoculated into the product, including *Saccharomyces cerevisiae*, *Escherichia coli*, and various *Leuconostoc* species. At the commercial scale, PEF treatments were observed to reduce both total aerobic microorganisms and yeasts and moulds by at least 6 log to  $<10$  CFU mL<sup>-1</sup> in both orange and tomato juice showing microbial shelf lives of 196 and 112 days, respectively, at 4°C (Min et al., 2003a, 2003b).

In addition to pore induction in microbial membranes, PEF can induce pore formation in the cells of plant material being processed for juice manufacture and several groups have exploited this capacity for the improvement of processing yields and efficiency. In apple juice production, the impact of PEF as an alternative to enzymatic mash maceration prior to pressing and juice collection has been examined by several laboratories. Perhaps due to the wide variation in experimental design, the results have been mixed, with some investigators demonstrating significant yield benefits following mash PEF treatment (Bazhal and Vorobiev, 2000; Lebovka et al., 2004; Schilling et al., 2007) while other researchers working at the pilot scale (220 kg apples) failed to demonstrate enhanced yields following mash treatments with either PEF or pectinases compared to untreated mash (Schilling et al., 2008). Despite the lack of consistent data regarding yields, PEF treatment may also provide benefits in terms of nutritional quality, where juices produced following PEF treatment of mash demonstrated enhanced antioxidant capacity and phenol content over juices produced by enzymatic maceration (Schilling et al., 2008). Such improvements were only observed under oxidative maceration treatments, suggesting that the higher phenol content of PEF-treated juices was a result of the reduced incubation time between mashing and pressing (Schilling et al., 2007).

As a nonthermal microcidal treatment, PEF processing has been demonstrated to protect key antioxidant nutrients in a range of juice products when compared against standard thermal pasteurization treatments. Following PEF treatment, strawberry, orange, and tomato juices retained close to 100% of the vitamin C content of untreated control juices. On the contrary, juices sterilized by heat treatment (90–95°C, 30–90 s) lost between 5% and 20% of initial ascorbic acid immediately following treatment (Yeom et al., 2000; Min et al., 2003a, 2003b; Odriozola-Serrano et al., 2008a). Furthermore, juices sterilized by PEF retained more ascorbic acid during subsequent storage than

either heat-treated or untreated juices such that orange juice retained 25 mg ascorbate per 100 mL (equivalent to the vitamin C RDA in a single serving) for between 14 and 16 days longer than heat-treated juice when stored at 4°C (Yeom et al., 2000; Min et al., 2003a). In the production of strawberry juice, PEF treatment also demonstrated benefits over conventional thermal treatments with regard to the maintenance of levels of phenolic compounds. PEF treatment resulted in a marginal, nonsignificant decline in total phenolic content compared to untreated juices, which was equivalent to that observed in juices that had been thermally processed at 90°C for 30 s. Increasing the thermal treatment period to 60 s resulted in a significant decline in the total phenol content from 47.3 mg 100 g<sup>-1</sup> to 43.6 mg 100 g<sup>-1</sup>. Both heat and PEF treatment resulted in a slower degradation of phenolic compounds than observed in untreated juice on storage, with PEF treatment being the most effective (Odriozola-Serrano et al., 2008a). The reasons for the slower degradation kinetics of both ascorbic acid and anthocyanins are unclear, although mechanisms may include the inactivation of oxidative or hydrolytic enzymes as has been demonstrated for lipoxygenase and β-glucosidase (Aguiló-Aguayo et al., 2009) and lower initial production of oxidizing compounds in PEF than in heat-treated juices. Q4

In addition to hydrophilic antioxidants, extensive research has been undertaken regarding the impact of PEF treatment on lipophilic antioxidants, with particular emphasis on carotenoids. Heat treatments have been found to have negative impacts on total carotenoid content in orange juice, with thermal treatment reducing total carotenoids and vitamin A by approximately 12.5% and 15.6%, respectively (Cortés et al., 2006a, 2006b). On the contrary, heat treatment only marginally reduced the lycopene content of tomato juice (<2%) and enhanced the concentration of both carotenoids (9%) and vitamin A (8%) in a 20% carrot–orange juice mix (Min et al., 2003b; Torregrosa et al., 2005). PEF treatments proved to be beneficial in comparison, resulting in only a 6.7% and 7.5% loss in carotenoids and vitamin A, respectively, from orange juice, a marginally improved retention of lycopene in tomato juice when compared with heat treatment, and significant further increases in carotenoid and vitamin A yields in carrot–orange juice over those observed for heat treatments. As observed for ascorbic acid, PEF treatments resulted in a greater retention of carotenoids and vitamin A on storage (Min et al., 2003a, 2003b; Cortés et al., 2006a). Analyses of changes in individual carotenoids following a range of PEF treatments (variable field strengths and treatment times) in orange and carrot–orange juice revealed varying effects on individual carotenoids. For example, treatment of orange juice at 35 or 40 kV cm<sup>-1</sup> resulted in improved recoveries of 13-*cis*-violaxanthin and 7,8,7',8'-tetrahydrolycopene while the concentrations of β-cryptoxanthin, phytoene, and β-carotene decreased (Cortés et al., 2006b). One mechanism proposed was PEF-induced *cis/trans* isomerization. Similar results were observed in carrot–orange juice mixtures; however, under appropriate conditions the concentrations of all carotenoids were increased, perhaps suggesting improved extraction and recovery prior to HPLC analysis as a result of PEF-induced macromolecular disruption (Torregrosa et al., 2005). This discussion highlights the requirement to define optimal PEF treatments for maximal nutritional benefit. A number of studies have addressed these issues, and it has been observed that while ascorbic acid concentration is best retained following low-intensity treatments (low field strengths, fewer, lower frequency and narrower pulses), higher phenolic and carotenoid retention is favored following intermediate- to high-intensity treatments (Odriozola-Serrano et al., 2007, 2008b, 2009; Oms-Oliu et al., 2009). Q5

### 25.3.2 HIGH HYDROSTATIC PRESSURES

High hydrostatic pressures (HHPs) represent an alternative method for the inactivation of microbial activity through the induction of membrane damage under reduced heating regimes, providing better retention of many antioxidant compounds (Knorr, 2003). It was originally developed as an alternative to pasteurization and sterilization for the reduction or removal of microbial activity (Guerrero-Beltran et al., 2005) and the inactivation of enzyme activities associated with loss of quality (Rastogi et al., 2007). For example, HHPs have been used to inactivate pectin methylesterase



in orange juice, thereby preventing the hydrolysis of pectin and maintaining the desirable cloud (Polydera et al., 2004). The fact that high-pressure-treated, extended shelf life products are available in Europe, the United States, and Japan is testament to the utility and cost effectiveness of the process (Butz et al., 2004; San Martin et al., 2002).

HHPs have been shown to be highly effective in reducing microbial activity in a range of fruit purees and juices. For example, the treatment of carrot and apple juices at 250 MPa (~25 atm) at 35°C for 15 min was sufficient to reduce the initial bacterial loads of 4.5–5.5 log CFU mL<sup>-1</sup> to below the limit of detection (<1 CFU mL<sup>-1</sup>) over subsequent storage for 30 days at 25°C (Dede et al., 2007). High-pressure treatments have also been demonstrated to be specifically effective against pathogenic bacteria, with *E. coli* O157 inactivated in apple and orange juices following treatment at 250 MPa for 20 min at 4°C or 25°C (Noma et al., 2004) and in apple, orange, apricot, and sour cherry juices following treatment at 250 MPa for 5 min at 40°C (Bayindirli et al., 2006). Bayindirli et al. (2006) also showed that other pathogenic microorganisms, including *Staphylococcus aureus* and *Salmonella enteritidis*, were inactivated under similar conditions; however, *Listeria monocytogenes* was more resistant to high pressures, requiring 300 and 400 MPa for 20 min for complete inactivation in peach and orange juice, respectively (Dogen and Erkmen, 2004). Given that commercial operation tends to be in excess of 400 MPa (Buzrul et al., 2008), the majority of vegetative cells are likely to be inactivated; however, spores are resistant to pressures up to 1200 MPa (Cheftel, 1995), requiring specific techniques such as high-pressure sterilization at temperatures in excess of 100°C for inactivation. Such techniques are known to negatively affect the functional properties of foods (Oey, et al., 2008).

The low operating temperatures usually applied under HHP processing result in protection of antioxidant vitamins and total antioxidant capacity in the material processed. Ascorbic acid shows little degradation under typical high-pressure processing conditions in citrus juices (Sánchez-Moreno et al., 2005). Similarly, ascorbic acid in noncitrus juices and nectars, including juices of carrot and tomato (Dede et al., 2007) and purees of guava (Yen and Lin, 1996) and strawberry (Sancho et al., 1999), was stable under high-pressure treatment. High-pressure treatment compared well with thermally processed juices, maintaining higher or equivalent levels of ascorbic acid in tomato, carrot, and orange juices (Bull et al., 2004; Dede et al., 2007; Sánchez-Moreno et al., 2005) and strawberry and guava purees (Sancho et al., 1999; Yen and Lin, 1996). Literature regarding the postprocessing stability of ascorbic acid in fruit juices presents mixed results, with some researchers reporting improved stability in pressure-treated juices compared with untreated or thermally treated juices (Dede et al., 2007; Yen and Lin, 1996) and others reporting few differences (Sancho et al., 1999; Bull et al., 2004). In one report, pressure-treated orange juice rapidly lost ascorbic acid on storage; however, no comparisons with other treatments were made (Nienaber and Shellhamer, 2001). Differences in the rates of ascorbic acid loss on storage could be attributed to the impact of microbial activity, packaging materials (García et al., 2001), or differences in gaseous headspace (Polydera et al., 2003).

Plant foods represent the most important contributor to dietary folate in the adult population; therefore, the impact of processing on stability and availability of folate is an important area of study (Scott et al., 2000). While there are a number of studies examining the kinetics of folate degradation in model systems, fewer reports are available concerning its stability under pressure in foods and juices. However, several forms of folic acid did show reasonable stability in orange juice following pressure treatment at 600 MPa for 5 min at 25°C: 5-methyl-tetrahydrofolate (5-methyl-THF) showed greater stability than 5-formyl-THF, which in turn was more stable than THF. Under these conditions all forms retained at least 80% of the content found in untreated juices; however, at a higher temperature of 80°C stability was reduced, and although more than 80% of the 5-methyl-THF was retained less than 80% and 70% THF and 5-formyl-THF were retained, respectively (Butz et al., 2004). Similar results were obtained by Indrawati et al. (2004), who observed a strong protective effect of ascorbic acid. In this study 5-methyl-THF was relatively baro- and thermostable in both orange juice and kiwi puree containing relatively high levels of ascorbic acid; however,

stability in carrot juice containing low levels of ascorbic acid was greatly reduced and the stability could be improved by its addition. High-pressure treatments had the additional benefit of increasing the bioavailability of folate in orange juice with an increased free 5-methyl-THF concentration following pressure treatments of 150 and 200 MPa (Indrawati et al., 2004).

In addition to analysis of the impact of high-pressure treatment on water-soluble vitamins, a number of studies have examined its impact on total antioxidant capacity. This parameter has normally been measured as the capacity of juices to scavenge either the 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS<sup>•+</sup>) or the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) and is generally reported as trolox equivalent antioxidant capacity (TEAC). Different authors have used reaction media of different polarity, affecting the antioxidant contribution from the lipophilic phase and making direct comparison between laboratories difficult; however, a number of generalizations can be made. Where direct comparisons have been undertaken, high pressure-treated tomato or carrot juice retained more antioxidant capacity than juices given a thermal treatment, resulting in equivalent microbial reduction (Dede et al., 2007). On the contrary, in a detailed kinetic analysis of the evolution of antioxidant capacity in thermally treated orange or carrot juice in the presence or absence of HHPs, an increase in antioxidant capacity following thermal treatment at atmospheric pressure that was substantially inhibited by high pressures was observed (Indrawati et al., 2004). However, the authors suggested that the increased antioxidant capacity observed under atmospheric pressure heating was a result of the generation of Maillard reaction products that had adverse effects on appearance and could potentially result in the formation of mutagenic compounds. For this reason, high-pressure processing (300–600 MPa) at mildly elevated temperatures (40–50°C) was recommended for the maintenance of antioxidant capacity and juice quality. Other authors failed to observe an improvement in the antioxidant capacity of thermally pasteurized orange juice (80°C, 60 s) and found that high-pressure-treated juice (600 MPa, 40°C, 4 min) had a higher initial antioxidant capacity that was also more stable to storage (Polydera et al., 2005). In one study, high-pressure treatment of orange juice significantly improved the recovery of the major flavonones naringenin and hesperetin, with treatment at 350 MPa for 2.5 min at 30°C improving recovery by 13% and 34%, respectively, against untreated juice (Sánchez-Moreno et al., 2003). Despite the improvement in flavonone recovery, total antioxidant capacity was unchanged from that of untreated juice, probably because the two flavonones combined accounted for less than 10% of the total antioxidant capacity of orange juice (Miller and Rice-Evans, 1997).

Regarding lipophilic antioxidants, several studies have demonstrated that high-pressure treatment improves the recovery of carotenoids in orange juice (de Ancos et al., 2002; Sánchez-Moreno et al., 2005) and persimmon puree (de Ancos et al., 2000), probably as a result of enhanced carotenoid release from denatured proteins and membranes. Enhancements of carotenoid recovery of up to 54% and 19% were observed in orange juice and persimmon puree, respectively. Recoveries of carotenoids were not linearly correlated with pressure, with low (50 MPa) and high (350 MPa) pressures resulting in greater carotenoid recovery from orange juice than intermediate pressures (de Ancos et al., 2002). These data may explain the lack of impact of high-pressure treatment on carotenoid recovery from orange juice treated at 600 MPa for 1 min (Bull et al., 2004).

In general, high-pressure treatment appears to be an effective way of controlling microbial activity in fruit juices without the loss of potentially health-beneficial antioxidants that are frequently unstable under thermal pasteurization and sterilization procedures. In addition, thermal treatments may actually improve the recovery and bioavailability of specific antioxidant compounds such as folates and carotenoids, which are found in close association with other macromolecules. Despite the useful data obtained to date, there are still several gaps in our knowledge regarding the impact of high-pressure treatments on a number of antioxidant compounds such as tocopherols and polyphenols. Future research should be geared toward filling in these important gaps in our knowledge.

### 25.3.3 DENSE PHASE CO<sub>2</sub>

The capacity of dense phase CO<sub>2</sub> to cause microbial inactivation was first observed in the 1950s (Fraser, 1951), although it was not until the late 1980s and early 1990s that researchers began to recognize its potential as a nonthermal, antimicrobial treatment for fruit juices. Continuous systems were introduced in the late 1990s, improving throughput and antimicrobial efficiency (Shimoda et al., 2001; Kincal et al., 2005). Two commercial systems were produced by Praxair and Air Liquide in the early 2000s (Damar and Balaban, 2006) and at least one system was installed by a US-based juice manufacturer (Anonymous, 2003), although it is currently unclear whether commercial systems are still available or whether any dense phase CO<sub>2</sub>-treated juices are marketed.

Dense phase CO<sub>2</sub> sterilization is undertaken at pressures significantly lower than those used for HPP treatment, being typically in the region of 5–30 MPa (Spilimbergo and Bertuccio, 2003). Several mechanisms have been proposed for microbial inactivation by dense phase CO<sub>2</sub>, including physical disruption of cells following pressure release, modification of cell membrane properties and permeability, reduction of cytoplasmic pH, inactivation of enzymes through the formation of arginine–bicarbonate complexes, and precipitation of intracellular Ca<sup>2+</sup> and Mg<sup>2+</sup> (Damar and Balaban, 2006). It appears that specific properties of CO<sub>2</sub> are responsible for microcidal action as other gases (nitrogen and argon) were ineffective when used under identical conditions; however, high-pressure nitrous oxide (N<sub>2</sub>O) has also been demonstrated to exhibit antimicrobial properties (Enomoto et al., 1997; Spilimbergo et al., 2007). Q8

The microcidal activity of dense phase CO<sub>2</sub> is comparable with pasteurization and performs favorably in comparison with other nonthermal technologies. In general, continuous systems show greater levels of inactivation than batch systems: for example, a 60 min treatment at 30 MPa was required to produce a 6 log reduction of *E. coli* in apple juice in a batch system (Liao et al., 2007), whereas 12 min at 20 MPa was sufficient to induce a similar reduction in a continuous system (Kincal et al., 2005). In addition to microcidal activity against bacteria, dense phase CO<sub>2</sub> has been demonstrated to be effective against fungi (Spilimbergo et al., 2007) and even highly resistant spores, where a greater than 6 log inactivation of *Saccharomyces cerevisiae* and *Alicyclobacillus acidoterrestris* spores was observed within 10 min of dense phase CO<sub>2</sub> treatment of orange juice (Sims and Estigarribia, 2002). It has been proposed that the high inactivation capacity of dense phase CO<sub>2</sub> against spores may be a result of its ability to cause germination (Furukawa et al., 2004). Q9

The few studies that have examined the impact of dense phase CO<sub>2</sub> treatment on the nutritional quality of juices have shown that antioxidant nutrients are generally well maintained and are not degraded as a result of treatment. In addition, the juices demonstrated improved storage stability. Likely mechanisms of protection include the inactivation of oxidative enzymes (Park et al., 2002) and the displacement of dissolved oxygen (Del Pozo-Insfran et al., 2006a). In muscadine grape juices treated with either dense phase CO<sub>2</sub> (34.5 MPa, 6.25 min, 8–16% CO<sub>2</sub>) or heat pasteurization (75°C, 15 s), the latter treatment caused an immediate loss of approximately 20% of anthocyanins, soluble phenolics, and antioxidant capacity and 10% of ascorbic acid compared with untreated juice while there were no significant differences between untreated juice and dense phase CO<sub>2</sub>-treated juice (Del Pozo-Insfran et al., 2006a, 2006b). Dense phase CO<sub>2</sub> reduced the yeast and mould counts by at least 5 log and microbiological stability was identical between CO<sub>2</sub> and heat-treated juice for at least 5 weeks (Del Pozo-Insfran et al., 2006a). Not only did CO<sub>2</sub>-treated juices display superior nutritional quality to thermally treated juices immediately following processing, but the rate of loss of anthocyanins, soluble phenolics, antioxidant capacity, and ascorbic acid was reduced on storage in CO<sub>2</sub>-treated juices. One potential problem identified was the acidification of juice caused by the dissolution of CO<sub>2</sub>; however, neither pH nor titratable acidity changed following CO<sub>2</sub> treatment and, furthermore, CO<sub>2</sub>-treated juices retained better organoleptic qualities than thermally treated juices. Similar experiments undertaken with apple juice using either dense phase CO<sub>2</sub> or N<sub>2</sub>O (batch process, 10 MPa, 10 min) leading to complete inactivation of spoilage microorganisms had no impact on the levels of ascorbic acid or total polyphenols, although there were

perceptible changes in juice quality and marked differences in a number of volatile aroma compounds (Gasperi et al., 2009).

#### 25.3.4 ULTRASOUND

Ultrasound has found a wide range of applications in the food industry, including nondestructive inspection, characterization of physicochemical properties (texture and viscosity), surface cleaning of foods and food processing equipment, enzyme inactivation, and ultrasound-assisted extraction, crystallization, emulsification, filtration, drying, and freezing (Knorr et al., 2004).

Like the PEF and HPP treatments, ultrasound treatments have been investigated for their capacity to inactivate microorganisms, which occurs by disruption to the cellular membrane caused by cavitation in which microbubbles are induced in the liquid. Subsequent collapse generates exceptionally high local temperatures (5500°C) and pressures (50 MPa). Despite these high local changes, through careful process control, bulk temperature elevation can be kept to a minimum, resulting in improved product quality and nutritional characteristics.

To date, experiments to determine the impacts of ultrasound on microbial inactivation have shown mixed results, with many studies suggesting that ultrasound in isolation will be insufficient to meet the 5 log reduction required by the US FDA. Thus Cheng et al. (2007) observed only marginal reductions of total microbial load from 4.05 to 3.94 log CFU mL<sup>-1</sup> in untreated and sonicated (35 kHz, 30 min) guava juice, respectively. Similarly, yeast and mould survival was only marginally reduced from 3.22 to 3.06 log CFU mL<sup>-1</sup>. As samples were treated in a commercially available sonic cleaning bath and no efforts were made to record energy input, it could be argued that treatments were insufficiently aggressive to have significant antimicrobial impact. However, detailed experiments analyzing a number of process parameters in both batch and continuous processing in orange juice also failed to confirm the utility of ultrasound for commercial pasteurization (Valero et al., 2007). In an initial series of experiments, juices were batch treated for 15 min at frequencies of either 500 or 23 kHz and power ratings between 120 and 600 W. All treatments caused a rise in temperature from 18°C to between 35°C and 88°C. Under these conditions, the maximum log reduction was 1.7 log CFU mL<sup>-1</sup> when using 23 kHz frequency at 600 W; however, this treatment also caused the maximum rise in temperature, which was shown to have a considerable impact on microorganisms in the absence of sonication. Treatment at high frequency using 240 W caused a 1.08 log decrease in total aerobic plate count while raising the temperature to only 51°C, a temperature shown to have a negligible impact on microorganisms alone. Clearly, the impact of sonication was well below the 5 log reduction required by the US FDA, and treatments were almost completely ineffective when considering moulds and yeasts. Suspended solids also had a negative impact on microcidal activity, where juice containing pulp added at a ratio of 1% or 10% prevented any microbial inactivation in a continuous system where ultrasound was applied to circulating juice for up to 180 min. Similarly, a variety of ultrasound treatments (up to 700 W, 60 min) were shown to be insufficiently effective at inactivating *Alicyclobacillus acidoterrestris*, a major spoilage organism in apple juice (Yuan et al., 2009). On the contrary, one study found sonication to be capable of reducing *E. coli* by up to 5 log cycles in apple cider, a traditional, nonalcoholic, minimally processed apple juice (Ugarte-Romero et al., 2006). In these experiments, apple cider was treated at a range of temperatures (40–60°C) with 20 kHz ultrasound and an acoustic energy density of 0.46 W mL<sup>-1</sup>. In the absence of sonication, *E. coli* inactivation at 40°C and 45°C was negligible and only a 0.67 log reduction was observed after 20 min at 50°C. Effective microbial inactivation was not achieved until temperatures were raised to 60°C, where a 5 log reduction was observed within 4 min. In the presence of sonication, *E. coli* inactivation kinetics were significantly enhanced at lower temperatures so that a 5 log reduction was achieved following around 15 min treatment at 40°C, 45°C, or 50°C or in just longer than 10 min at 55°C. At 60°C, sonication had little impact on inactivation kinetics and cell inactivation was only increased by 0.1 log cycles over temperature treatment alone.

An alternative to ultrasound-induced cavitation is hydrodynamic cavitation induced by subjecting liquid foods to extreme flow conditions. In one experiment in which cavitation was induced by subjecting juices to mixing in a high-speed rotor, a 5-log reduction of *Lactobacillus plantarum*, *Lactobacillus sakei*, and *Zygosaccharomyces bailii* in apple juice was achieved along with a concomitant rise in temperature up to 77° (Milly et al., 2007). Taken together, the available data suggest that while ultrasound may be insufficient alone to achieve the microbial reduction required, combination with other treatments may offer an opportunity to reduce the harshness of microbial inactivation treatments, thereby improving the nutritional quality of juices.

Although several studies have analyzed the impact of sonication on juice quality, direct comparisons with alternative sterilization technologies (e.g., thermal treatments) are limited as are studies that combine analysis of juice quality with impact on microbial inactivation. In one study, treatment of guava juice in an ultrasonic cleaning bath at 35 kHz resulted in improved ascorbic acid content. Moreover, it was suggested that sonication caused the elimination of dissolved oxygen, resulting in lower oxidative degradation (Cheng et al., 2007). However, the same study showed that PPO activity, a key enzyme implicated in reduced nutritional and sensory quality as a result of enzymatic browning, was increased. Furthermore, the treatment conditions used were only marginally effective for microbial activation. In recent years, a group based at University College Dublin has been particularly active in examining the impact of ultrasound on the nutritional quality of juices. Ascorbic acid degradation was observed to be dependent on treatment time and acoustic energy density when treated at a constant frequency of 20 kHz in both strawberry (Tiwari et al., 2009a) and orange juice (Tiwari et al., 2009b), although maximal losses were below 15% and 5%, respectively. Sonicated orange juice samples had slower ascorbic acid degradation kinetics on storage, although the same effect was not observed in strawberry juices. Anthocyanins were found to be more resistant to ultrasound-induced degradation than ascorbic acid in both strawberry and blackcurrant juice, exhibiting a maximum loss of 5% (Tiwari et al., 2009a, 2009c). Both increased acoustic energy density and treatment time resulted in greater degradation; however, at low treatment times and energy densities an enhancement of anthocyanin content was observed in strawberry juice, probably as a result of anthocyanin release from suspended pulp particles.

At present, only limited data are available to allow assessment of the potential for ultrasound in juice processing. Although ultrasound appears to cause low losses of important nutritional components, further comparative studies are required. However, the mixed results regarding microbial inactivation, the potential energy inputs required, and the difficulty in designing high-throughput continuous systems suitable for commercial-scale processing suggest that it is likely to lag behind other alternatives to thermal pasteurization.

### 25.3.5 ULTRAVIOLET LIGHT

Ultraviolet (UV) light is currently commercially used as an alternative to thermal pasteurization in the production of a number of fruit juices, including apple cider produced in Northeast America (Hanes et al., 2002) and a number of juices produced in South Africa (Keyser et al., 2008). One of the most appealing aspects concerning the use of UV is its potential microcidal activity at low temperatures; however, the use of UV in the processing of fruit juices has not received the same attention as some other potential processes. Some literature is available regarding its germicidal activity in fruit juices, and a small number of papers dealing with its impact on quality and nutritional parameters will be outlined here. An excellent recent review provides further details regarding theory and practical considerations for the UV treatment of juices (Koutchma, 2009).

One problem associated with UV inactivation of microbes in fruit juices is related to the high absorbance coefficients of the products, resulting in 90% absorbance as light penetrates into the first 10–100  $\mu\text{m}$  of juice. This problem has been overcome by reactor design in which juices are exposed to light either under a narrow laminar flow or under conditions of high turbulence, where the juice is rapidly mixed resulting in all parts being exposed to the UV-producing surface. Laminar flow

systems were shown to effectively reduce the loads of *E. coli*, *Listeria innocua*, and the protozoan parasite *Cryptosporidium parvum* by at least 5 log cycles in apple cider; however, microcidal activity against *Saccharomyces cerevisiae* was not as effective, causing only a 1.34 log reduction under equivalent process conditions (Hanes et al., 2002; Guerrero-Beltrán and Barbosa-Cánovas, 2005). The inefficiency of UV treatment in reducing yeast and mould numbers has also been reported elsewhere (Choi and Nielsen, 2005) and it has been suggested that hot filling (63°C) of apple cider bottles is more effective at reducing their numbers (Tandon et al., 2003). Similarly, UV was less efficient in reducing yeasts and mould (~2 log reduction) than total aerobes (~3 log reduction) in orange juice (Tran and Farid, 2004). The impact of juice type and UV dose was examined in a turbulent system by Keyser et al. (2008), who did not observe consistent differences between the susceptibility of total aerobes and yeasts and moulds but did observe a strong reduction in microcidal activity in turbid (orange and tropical mix) or concentrated (strawberry and mango nectar) compared with less turbid juices (apple, guava, and pineapple).

Nutritional components with absorption maxima close to the UV output maximum (~254 nm) of current commercial systems are those most likely to suffer detrimental effects from UV treatment. These include ascorbic acid with maximal absorbances at 243 and 265 nm in acid and neutral media, respectively (Hancock et al., 2008), a number of carotenoids that have absorption maxima in the range 240–280 nm (Du et al., 1998), and several classes of phenolic compounds such as flavonoids, which include the important color-providing anthocyanins with absorption maxima in the range 250–275 nm (Harborne, 1989). Such predictions were confirmed following UV treatment of orange juice, where losses of  $\beta$ -carotene and riboflavin were close to 50% while ascorbic acid losses were in the region of 20% (Koutchma, 2009).

### 25.3.6 IONIZING RADIATION

A number of authors have investigated the impact of ionizing radiation on the microbiological safety and nutritional quality of fruit juices. While irradiation was shown to be highly effective in reducing microbial contamination in a range of juices (Buchanan et al., 1998; Wang et al., 2006; Song et al., 2007), it has been demonstrated to have negative impacts on ascorbic acid content (Fan et al., 2002; Song et al., 2007), anthocyanin content (Alighourchi et al., 2008), and organoleptic quality (Wang et al., 2006; Song et al., 2007). In addition, ionizing radiation has been shown to promote the formation of potentially toxic aldehydes (formaldehyde, acetaldehyde, and malondialdehyde) in apple juice (Fan and Thayer, 2002). For these reasons, irradiation of fruit juices is unlikely to provide any nutritional benefits over standard pasteurization technologies.

## 25.4 CONCLUSIONS

As consumers continue to seek products with improved nutritional value and functionality, juice producers have the opportunity of improving product marketability through the use of novel technologies that maintain levels of key phytonutrients. Indeed, many juices are currently marketed for their proposed nutritional benefits, including many brands that claim high antioxidant capacity (e.g., POM Wonderful pomegranate juice and Ribena blackcurrant juice). Laboratory and pilot-scale research have demonstrated the advantages of nonthermal extraction and pasteurization technologies in maximizing the nutritional value and fresh qualities of fruit juices; however, with the exception of enzyme-aided extraction it remains unclear which, if any, of the technologies can provide sufficient advantage at an economically viable cost.

As food labeling and nutritional claims rules become stricter (e.g., European Union regulation 1924/2006), it will become necessary to provide further evidence in support of ascribed functional properties and simple statements such as “high in antioxidants” are unlikely to be sufficient. Indeed, there is a growing consensus within the scientific community that antioxidant capacity *per se* is not

Q10

relevant to the health benefits of fruit and vegetable consumption and, in fact, pharmacological interactions with individual compounds that happen to have a high antioxidant capacity are more likely mediating such benefits (Stevenson and Lowe, 2009). In future it may therefore be necessary to conduct more detailed studies in which the impact of processing on individual compounds and their chemical transformations are examined.

## REFERENCES

- Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R., and Martín-Belloso, O., 2009. Flavour retention and related enzyme activities during storage of strawberry juices processed by high-intensity pulsed electric fields or heat. *Food Chem.* 116: 59–65.
- Alighourchi, H., Barzegar, M., and Abbasi, S., 2008. Effect of gamma irradiation on the stability of anthocyanins and shelf-life of various pomegranate juices. *Food Chem.* 110: 1036–1040.
- de Ancos, B., Gonzalez, E., and Cano, M.P., 2000. Effect of high-pressure treatment on the carotenoid composition and radical scavenging activity of persimmon fruit purees. *J. Agric. Food Chemistry* 48: 3542–3548.
- de Ancos, B., Sgroppo, S., Plaza, L., and Cano, M.P., 2002. Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *J. Sci. Food Agric.* 82: 790–796.
- Anonymous. 2003. Sun orchard to install non-thermal juice processing system from Praxair. Praxair press release. Available at <http://www.praxair.com/praxair.nsf/AllContent/9161E80611AE461E85256CE1006A1633?OpenDocument>. Accessed July 1, 2009.
- Bagger-Jørgensen, R. and Meyer, A.S., 2004. Effects of different enzymatic pre-press maceration treatments on the release of phenols into blackcurrant juice. *Eur. Food Res. Technol.* 219: 620–629.
- Bayindirli, A., Alpas, H., Bozoğlu, F., and Hizal, M., 2006. Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. *Food Control* 17: 52–58.
- Bazhal, M. and Vorobiev, E., 2000. Electrical treatment of apple cassettes for intensifying juice processing. *J. Sci. Food Agric.* 80: 1668–1674.
- Bjelakovic, G., Nikolova, D., Glud, L.L., Simonetti, R.G., and Glud, C., 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. Systematic review and meta-analysis. *J. Am. Med. Assoc.* 297: 842–857.
- Block, G., Patterson, B., and Subar, A., 1992. Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer* 18: 1–29.
- Bub, A., Watzl, B., Blockhaus, M., Briviba, K., Liegibel, U., Müller, H., Pool-Zobel, B.L., and Reckemmer, G., 2003. Fruit juice consumption modulates antioxidative status, immune status and DNA damage. *J. Nutr. Biochem.* 14: 90–98.
- Buchanan, R.L., Edelson, S.G., Snipes, K., and Boyd, G., 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. *Applied and Environmental Microbiology* 64: 4533–4535.
- Buchert, J., Koponen, J.M., Suutarinen, M., Mustranta, A., Lille, M., Törrönen, R., and Poutanen, K., 2005. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* 85: 2548–2556.
- Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Sockman R., Sellahewa J., Szabo, E.A., Johnson, R.L., and Stewart, C.M., 2004. The effect of high pressure processing in the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies* 5: 135–149.
- Butz, P., Serfert, Y., Garcia, A.F., Dieterich, S., Lindauer, R., Bogner, A., and Tauscher, B., 2004. Influence of high-pressure treatment at 25°C and 80°C on folates in orange juice and model media. *Journal of Food Science* 69: 117–121.
- Buzrul, S., Alpas, H., Largeteau, A., and Demazeau, G., 2008. Inactivation of *Escherichia coli* and *Listeria innocua* in kiwifruit and pineapple juices by high hydrostatic pressure. *International Journal of Food Microbiology* 142: 275–278.
- Chang, D.C. and Reese, T.S., 1990. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophysical Journal* 58: 1–12.
- Cheftel, J.C., 1995. Review: High-pressure microbial inactivation and food processing. *Food Science and Technology International* 1: 75–90.
- Cheng, L.H., Soh, C.Y., Liew, S.C., and The, F.F., 2007. Effects of sonication and carbonation on guava juice quality. *Food Chem.* 104: 1396–1401.

- Choi, L.H. and Nielsen, S.S., 2005. The effects of thermal and nonthermal processing methods on apple cider quality and consumer acceptability. *Journal of Food Quality* 28: 13–29.
- Cortés, C., Esteve, M.J., Rodrigo, D., Torregrosa, F., and Frígola, A., 2006b. Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology* 44: 1932–1939.
- Cortés, C., Torregrosa, F., Esteve, M.J., and Frígola, A., 2006a. Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with high-intensity pulsed electric fields. *J. Agric. Food Chem.* 54: 6247–6254.
- Damar, S. and Balaban, M.O., 2006. Review of dense phase CO<sub>2</sub> technology: Microbial and enzyme inactivation, and effects on food quality. *Journal of Food Science* 71: R1–R11.
- Dede, S., Alpas, H., and Bayindirli A., 2007. High hydrostatic pressure treatment and storage of carrot and tomato juices: Antioxidant activity and microbial safety. *J. Sci. Food Agric.* 87: 773–782.
- Del Pozo-Insfran, D., Balaban, M.O., and Talcott, S.T., 2006a. Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO<sub>2</sub> processed muscadine grape juice. *J. Agric. Food Chem.* 54: 5468–5473.
- Del Pozo-Insfran, D., Balaban, M.O., and Talcott, S.T., 2006b. Enhancing the retention of phytochemicals and organoleptic attributes in muscadine grape juice through a combined approach between dense phase CO<sub>2</sub> processing and copigmentation. *J. Agric. Food Chem.* 54: 6705–6712.
- Diplock, A.T., Charleux, J-L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W., and Viña-Ribes, J., 2003. Functional food science and defence against reactive oxygen species. *Brit. J. Nutr.* 80: S77–S112.
- Dogen, C. and Erkmén, O., 2004. High pressure inactivation kinetics of *Listeria monocytogenes* inactivation in broth, milk, and peach and orange juices. *J. Food Eng.* 62: 47–52.
- Du, H., Fuh, R.A., Li, J., Corkan, A., and Lindsey, J.S., 1998. PhotochemCAD: A computer-aided design and research tool in photochemistry. *Photochem. Photobiol.* 68: 141–142.
- Dunn, J.E. and Pearlman, J.S., 1987. Methods and apparatus for extending the shelf life of fluid food products. US Patent 4 695 472.
- Enomoto, A., Nakamura, K., Nagai, K., Hashimoto, T., and Hakoda, M., 1997. Inactivation of food microorganisms by high-pressure carbon dioxide treatment with or without explosive decompression. *Biosci., Biotechnol. Biochem.* 61: 1133–1137.
- Fan, X. and Thayer, D.W., 2002. Formation of malondialdehyde, formaldehyde, and acetaldehyde in apple juice induced by ionizing radiation. *J. Food Sci.* 67: 2523–2528.
- Fan, X., Thayer, D.W., and Handel, A.P., 2002. Nutritional quality of irradiated orange juice. *J. Food Process. Pres.* 26: 195–211.
- Favela-Torres, E., Volke-Sepúlveda, T., and Viniegra-González, G., 2006. Production of hydrolytic depolymerizing pectinases. *Food Technol. Biotechnol.* 44: 221–227.
- Fraser, D., 1951. Bursting bacteria by release of gas pressure. *Nature* 167: 33–34.
- Freidman, M.A. and Shalala, D.E., 1998. Food labeling: Warning and notice statement; labeling of juice products. *Federal Register* 63: 37030–37056.
- Furukawa, S., Watanabe, T., Tai, T., Hirata, J., Narisawa, N., Kawarai, T., Ogihara, H., and Yamasaki, M., 2004. Effect of high pressure CO<sub>2</sub> on the germination of bacterial spores. *Int. J. Food Microbiol.* 91: 209–213.
- García, A.F., Butz, P., Bognár, A., and Tauscher, B., 2001. Antioxidative capacity, nutrient content and sensory quality of orange juice and an orange-lemon-carrot juice product after high pressure treatment and storage in different packaging. *Eur. Food Res. Technol.* 213: 290–296.
- García-Alonso, J., Ros, G., Vidal-Guevara, M.L., and Periago, M.J., 2006. Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. *Nutr. Res.* 26: 330–339.
- Gasperi, F., Aprea, E., Biasioli, F., Carlin, S., Endrizzi, I., Pirretti, G., and Spilimbergo, S., 2009. Effects of supercritical CO<sub>2</sub> and N<sub>2</sub>O pasteurisation on the quality of fresh apple juice. *Food Chem.* 115: 129–136.
- Goldberg, J., Flowerdew, G., Smith, E., Brody, J.A., and Tso, M.O.M., 1988. Factors associated with age-related macular degeneration: An analysis of data from the first national health and nutrition examination survey. *Am. J. Epidemiol.* 128: 700–710.
- Grahl, T. and Markl, H., 1996. Killing of microorganisms by pulsed electric fields. *Appl. Microbiol. Biotechnol.* 67: 148–157.
- Guerrero-Beltrán, J.A. and Barbosa-Cánovas, G.V., 2005. Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *J. Food Process Eng.* 28: 437–452.
- Guerrero-Beltrán, J.A., Barbosa-Cánovas, G.V., and Swanson, B.G., 2005. High hydrostatic pressure processing of fruit and vegetable products. *Food Rev. Int.* 21: 411–425.



- Hancock, R.D., Chudek, J.A., Walker, P.G., Pont, S.D.A., and Viola, R., 2008. Ascorbic acid conjugates isolated from the phloem of *Cucurbitaceae*. *Phytochemistry* 69: 1850–1858.
- Hancock, R.D., McDougall, G.J., and Stewart, D., 2007. Berry fruit as ‘superfood’: Hope or hype? *The Biologist* 54: 73–79.
- Hanes, D.E., Worobo, R.W., Orlandi, P.A., Burr, D.H., Miliotis, M.D., Robl, M.G., Bier, J.W., Arrowood, M.J., Churey, J.J., and Jackson, G.J., 2002. Inactivation of *Cryptosporidium parvum* oocysts in fresh apple cider by UV irradiation. *Appl. Environ. Microbiol.* 68: 4168–4172.
- Harborne, J.R., 1989. General procedures and measurement of total phenolics. In: J.B. Harborne (Ed.), *Methods in Plant Biochemistry. Volume 1, Plant Phenolics*, pp. 1–28. London: Academic Press.
- Harrison, S.L., Barbosa-Cánovas, G.V., and Swanson, B.G., 1997. *Saccharomyces cerevisiae* structural changes induced by pulsed electric field treatment. *Lebensm. Wiss. Technol.* 30: 236–240.
- Henderson, L., Gregory, J., and Swan, G., 2002. *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. London: HMSO.
- Hilz, H., Bakx, E.J., Schols, H.A., and Voragen, A.G.J., 2005. Cell wall polysaccharides in black currants and bilberries—characterisation in berries, juice, and press cake. *Carbohydr. Polym.* 59: 477–488.
- Indrawati, Arroqui, C., Messagie, I., Nguyen, M.T., van Loey A., and Hendrickx, M., 2004. Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. *J. Agric. Food Chem.* 52: 485–492.
- Jayani, R.V., Saxena S., and Gupta R., 2005. Microbial pectinolytic enzymes: A review. *Process Biochem.* 40: 2931–2944.
- Kashyap, D.R., Vohra, P.K., Chopra, S., and Tewari, R., 2001. Applications of pectinases in the commercial sector: A review. *Bioresource Technol.* 77: 215–227.
- Keyser, M., Müller, I.A., Cilliers, F.P., Nel, W., and Gouws, P.A., 2008. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innov. Food Sci. Emer. Technol.* 9: 348–354.
- Kincal, D., Hill, W.S., Balaban, M.O., Portier, K.M., Wei, C.I., and Marshall, M.R., 2005. A continuous high pressure carbon dioxide system for microbial reduction in orange juice. *J. Food Sci.* 70: M249–M254.
- Knorr, D. 2003. Impact of non-thermal processing on plant metabolites. *J. Food Eng.* 56: 131–134.
- Knorr, D., Zenker, M., Heinz, V., and Lee, D.-U., 2004. Applications and potential of ultrasonics in food processing. *Trends Food Sci. Technol.* 15: 261–266.
- Koponen, J.M., Buchert, J., Poutanen, K.S., and Törrönen, A.R., 2008. Effect of pectinolytic juice production on the extractability and fate of bilberry and black currant anthocyanins. *Eur. Food Res. Technol.* 227: 485–494.
- Koutchma, T., 2009. Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food Bioprocess Technol.* 2: 138–155.
- Landbo, A.-K., Kaack, K., and Mayer, A.S., 2007. Statistically designed two step response surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice. *Innov. Food Sci. Emer. Technol.* 8: 135–142.
- Landbo, A.-K. and Meyer, A.S., 2004. Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice. *Innov. Food Sci. Emer. Technol.* 5: 503–513.
- Landbo, A.-K.R., Pinelo, M., Vikbjerg, A.F., Let, M.B., and Meyer, A.S., 2006. Protease-assisted clarification of black currant juice: Synergy with other clarifying agents and effects on the phenol content. *J. Agric. Food Chem.* 54: 6554–6563.
- La Vecchia, C., Decarli, A., and Pagano, R., 1998. Vegetable consumption and risk of chronic disease. *Epidemiology* 9: 208–210.
- Lebovka, N.I., Praporscic, I., and Vorobiev, E., 2004. Combined treatment of apples by pulsed electric fields and by heating at moderate temperature. *J. Food Eng.* 65: 211–217.
- Liao, H., Hu, X., Liao, X., Chen, F., and Wu, J., 2007. Inactivation of *Escherichia coli* inoculated into cloudy apple juice exposed to dense phase carbon dioxide. *Int. J. Food Microbiol.* 118: 126–131.
- Liu, X., Yousef, A.E., and Chism, G.W., 1997. Inactivation of *Escherichia coli* O157:H7 by the combination of organic acids and pulsed electric fields. *J. Food Safety* 16: 287–299.
- Mares-Perlman, J., Brady, W.E., Klein, B.E., Klein, R., Haus, G.J., Palta, M., Ritter, L.L., and Shoff, S.M., 1995. Diet and nuclear lens opacities. *Am. J. Epidemiol.* 141: 322–334.
- McDougall, G.J., Gordon, S., Brennan, R., and Stewart, D., 2005. Anthocyanin-flavanol condensation products from black currant (*Ribes nigrum* L.). *J. Agric. Food Chem.* 53: 7878–7885.

- McGhie, T.K., Walton, M.C., Barnett, L.E., Vather, R., Martin, H., Au, J., Alspach, P.A., Booth, C.L., and Kruger, M.C., 2007. Boysenberry and blackcurrant drinks increased the plasma antioxidant capacity in an elderly population but had little effect on other markers of oxidative stress. *J. Sci. Food Agric.* 87: 2519–2527.
- Mihalev, K., Schieber, A., Mollov, P., and Carle, R., 2004. Effect of mash maceration on the polyphenolic content and visual quality attributes of cloudy apple juice. *J. Agric. Food Chem.* 52: 7306–7310.
- Miller, N.J. and Rice-Evans, C.A., 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.* 60: 331–337.
- Milly, P.J., Toledo, R.T., Harrison, M.A., and Armstead, D., 2007. Inactivation of food spoilage microorganisms by hydrodynamic cavitation to achieve pasteurization and sterilization of fluid foods. *J. Food Sci.* 72: M414–M422.
- Min, S., Evrendilek, G.A., and Zhang, Q.H., 2007. Pulsed electric fields: Processing system, microbial and enzyme inhibition, and shelf life extension of foods. *IEEE Trans. Plasma Sci.* 35: 59–73.
- Min, S., Jin, Z.T., Yeom, H.W., Min, S.K., and Zhang, Q.H., 2003a. Commercial-scale pulsed electric field processing of orange juice. *J. Food Sci.* 68: 1265–1271.
- Min, S., Jin, Z.T., and Zhang, Q.H., 2003b. Commercial scale pulsed electric field processing of tomato juice. *J. Agric. Food Chem.* 51: 3338–3344.
- Min., S., Reina, L., and Zhang, Q.H., 2002. Water activity and the inactivation of *Enterobacter cloacae* inoculated in chocolate liquor and a model system by pulsed electric field treatment. *J. Food Process. Preserv.* 26: 323–337.
- Ness, A.R. and Powles, J.W., 1997. Fruit and vegetables, and cardiovascular disease: A review. *Int. J. Epidemiol.* 26: 1–13.
- Netzel, M., Strass, G., Kaul, C., Bitsch, I., Dietrich, H., and Bitsch, R., 2002. *In vivo* antioxidative capacity of a composite berry juice. *Food Res. Int.* 35: 213–216.
- Nienaber, U. and Shellhammer, T.H., 2001. High-pressure processing of orange juice: Combination treatments and a shelf life study. *J. Food Sci.* 66: 332–336.
- Noma, S., Tomita C., Shimoda M., and Hayakawa, I., 2004. Response of *Escherichia coli* O157:H7 in apple and orange juices by hydrostatic pressure treatment with rapid decompression. *Food Microbiol.* 21: 469–473.
- Odrizola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., and Martín-Belloso, O., 2007. Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *J. Agric. Food Chem.* 55: 9036–9042.
- Odrizola-Serrano, I., Soliva-Fortuny, R., Gimeno-Añó, V., and Martín-Belloso, O., 2008b. Kinetic study of anthocyanins, vitamin C, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields. *J. Agric. Food Chem.* 56: 8387–8393.
- Odrizola-Serrano, I., Soliva-Fortuny, R., and Martín-Belloso, O., 2008a. Phenolic acids, flavonoids, vitamin C and antioxidant capacity of strawberry juices processed by high-intensity pulsed electric fields or heat treatments. *Eur. Food Res. Technol.* 228: 239–248.
- Odrizola-Serrano, I., Soliva-Fortuny, R., and Martín-Belloso, O., 2009. Impact of high-intensity pulsed electric fields variables in vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT—Food Sci. Technol.* 42: 93–100.
- Oey, I., Van der Plancken, I., Van Loey, A., and Hendrickx, M., 2008. Does high pressure processing influence nutritional aspects of plant based food systems? *Trends Food Sci. Technol.* 19: 300–308.
- Oms-Oliu, G., Odrizola-Serrano, I., Soliva-Fortuny, R., and Martín-Belloso, O., 2009. Effects of high-intensity pulsed electric field processing conditions on lycopene, vitamin C and antioxidant capacity of watermelon juice. *Food Chem.* 115: 1312–1319.
- Park, S.-J., Lee, J.-I., and Park, J., 2002. Effects of combined process of high pressure CO<sub>2</sub> and high hydrostatic pressure on the quality of carrot juice. *J. Food Sci.* 67: 1827–1833.
- Polydera, A.C., Galanou, E., Stoforos, N.G., and Taoukis, P.S., 2004. Inactivation kinetics of pectin methyl-esterase of greek Navel orange juice as a function of high hydrostatic pressure and temperature process conditions. *J. Food Eng.* 62: 291–298.
- Polydera, A.C., Stoforos, N.G., and Taoukis, P.S., 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *J. Food Eng.* 60: 21–29.
- Polydera, A.C., Stoforos, N.G., and Taoukis, P.S., 2005. Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice. *Food Chem.* 91: 495–503.
- Pothakamury, U.R., Vega, H., Zhang, Q., Barbosa-Cánovas, G.V., and Swanson, B.G., 1996. Effect of growth stage and processing temperature on the inactivation of *E. coli* by pulsed electric fields. *J. Food Protect.* 59: 1167–1171.

- Puupponen-Pimiä, R., Nohynek, L., Ammann, S., Oksman-Caldentey, K.-M., and Buchert, J., 2008. Enzyme-assisted processing increases antimicrobial and antioxidant activity of bilberry. *J. Agric. Food Chem.* 56: 681–688.
- Rastogi, N.K., Raghavarao, K.S.M.S., Balasubramaniam, V.M., Niranjana, K., and Knorr, D., 2007. Opportunities and challenges in high pressure processing of foods. *Crit. Rev. Food Sci. Nutr.* 47: 69–112.
- Riihinen, K., Jaakola, L., Kärenlampi, S., and Hohtola A., 2008. Organ-specific distribution of phenolic compounds in bilberry (*Vaccinium myrtillus*) and ‘northblue’ blueberry (*Vaccinium corymbosum* x *V. angustifolium*). *Food Chem.* 110: 156–160.
- Ristow, M., Zarse, K., Oberbach, A., Klötting, N., Birringer, M., Kiehnopf, M., Stumvoli, M., Kahn, C.R., and Blüher, M., 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl. Acad. Sci. USA* 106: 8665–8670.
- Roodenburg, B., Morren, J., Berg, H.E., and de Haan, S.W.H., 2005. Metal release in a stainless steel pulsed electric field (PEF) system. Part II. The treatment of orange juice; related to legislation and treatment chamber lifetime. *Innov. Food Sci. Emer. Technol.* 6: 337–345.
- Ros Barcelo, A., Calderon, A.A., Zapata, J.M., and Muñoz, R., 1994. The histochemical localization of anthocyanins in seeded and seedless grapes (*Vitis vinifera*). *Scientia Horticulturae* 57: 265–268.
- Sadilova, E., Stintzing, F.C., and Carle, R., 2006. Thermal degradation of acylated and nonacylated anthocyanins. *J. Food Sci.* 71: C504–C512.
- Sánchez-Moreno, C., Plaza, L., de Ancos, B., and Cano, M.P., 2003. Effect of high-pressure processing on health-promoting attributes of freshly squeezed orange juice (*Citrus sinensis* L.) during chilled storage. *Eur. Food Res. Technol.* 216: 18–22.
- Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., and Cano, M.P., 2005. Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *J. Agric. Food Chem.* 53: 4403–4409.
- Sancho, F., Lambert, Y., Demazeau, G., Largeteau, A., Bouvier, J.-M., and Narbonne, J.-F., 1999. Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. *J. Food Eng.* 39: 247–253.
- San Martín, M.F., Barbosa-Cánovas, G.V., and Swanson, B.G., 2002. Food processing by high hydrostatic pressure. *Crit. Rev. Food Sci. Nutr.* 42: 627–645.
- Schilling, S., Alber, T., Toepfl, S., Neidhart, S., Knorr, D., Scieber, A., and Carle R., 2007. Effects of pulsed electric field treatment of apple mash on juice yield and quality attributes of apple juices. *Innov. Food Sci. Emer. Technol.* 8: 127–134.
- Schilling, S., Toepfl, S., Ludwig, M., Dietrich, H., Knorr, D., Neidhart, S., Schieber, A., and Carle, R., 2008. Comparative study of juice production by pulsed electric field treatment and enzymatic maceration of apple mash. *Eur. Food Res. Technol.* 226: 1389–1398.
- Scott, J., Rébeille, F., and Fletcher, J., 2000. Review: Folic acid and folates: The feasibility for nutritional enhancement in plant foods. *J. Sci. Food Agric.* 80: 795–824.
- Selman, C., McLaren, J.S., Meyer, C., Duncan, J.S., Redman, P., Collins, A.R., Duthie, G., and Speakman, J.R., 2006. Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mech. Ageing Develop.* 127: 897–904.
- Semenova, M.V., Sinitsyna, O.A., Morozova, V.V., Fedorova, E.A., Gusakov, A.V., Okunev, O.N., Sokolova, L.M. et al., 2006. Use of a preparation from fungal pectin lyase in the food industry. *Appl. Biochem. Microbiol.* 42: 681–685.
- Shimoda, M., Cocunubo-Castellanos, J., Kago, H., Miyake, M., Osajima, Y., and Hayakawa, I., 2001. The influence of dissolved CO<sub>2</sub> concentration on the death kinetics of *Saccharomyces cerevisiae*. *J. Appl. Microbiol.* 91: 306–311.
- Siebert, K.J., 1999 Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *J. Agric. Food Chem.* 47: 353–363.
- Sims, M. and Estigarribia, E., 2002. Continuous sterilization of aqueous pumpable food using high pressure CO<sub>2</sub>. *Chem. Eng. Trans.* 2: 921–926.
- Song, H.-P., Byun, M.-W., Jo, C., Lee, C.-H., Kim, K.-S., and Kim, D.-H., 2007. Effects of gamma irradiation on the microbiological, nutritional, and sensory properties of fresh vegetable juice. *Food Control* 18: 5–10.
- Spilimbergo, S. and Bertucco, A., 2003. Non-thermal bacteria inactivation with dense CO<sub>2</sub>. *Biotechnol. Bioeng.* 84: 627–638.
- Spilimbergo, S., Mantoan, D., and Dalser, A., 2007. Supercritical gases pasteurization of apple juice. *J. Supercrit. Fluids* 40: 485–489.











- Stevenson, D.E. and Hurst, R.D., 2007. Polyphenolic phytochemicals—just antioxidants or much more? *Cell. Mol. Life Sci.* 64: 2900–2916.
- Stevenson, D.E. and Lowe T., 2009. Plant-derived compounds as antioxidants for health—are they all really antioxidants? *Funct. Plant Sci. Biotechnol.* (in press).
- Tandon, K., Worobo, R.W., Churey, J.J., and Padilla-Zakour, O.I., 2003. Storage quality of pasteurized and UV treated apple cider. *J. Food Process. Preserv.* 27: 21–35.
- Tiwari, T.K., O'Donnell, C.P., and Cullen, P.J., 2009c. Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends Food Sci. Technol.* 20: 137–145.
- Tiwari, B.K., O'Donnell, C.P., Muthukumarappan, K., and Cullen, P.J., 2009b. Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT—Food Sci. Technol.* 42: 700–704.
- Tiwari, B.K., O'Donnell, C.P., Patras, A., Brunton, N., and Cullen, P.J., 2009a. Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. *Eur. Food Res. Technol.* 228: 717–724.
- Torregrosa, F., Cortés, C., Esteve, M.J., and Frígola, A., 2005. Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *J. Agric. Food Chem.* 53: 9519–9525.
- Tran, M.T.T. and Farid, M., 2004. Ultraviolet treatment of orange juice. *Innov. Food Sci. Emer. Technol.* 5: 495–502.
- Ugarte-Romero, E., Feng, H., Martin, S.E., Cadwallader, K.R., and Robinson, S.J., 2006. Inactivation of *Escherichia coli* with power ultrasound in apple cider. *J. Food Sci.* 71: E102–E108.
- Valero, M., Recrosio, N., Saura, D., Muñoz, N., Martí, N., and Lizama, V., 2007. Effects of ultrasonic treatments in orange juice processing. *J. Food Eng.* 80: 509–516.
- van der Sluis, A.A., Dekker, Matthus, D., Skrede, G., and Jongen, W.M.F., 2002. Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *J. Agric. Food Chem.* 50: 7211–7219.
- Vega-Mercado, H., Pothakamury, U.R., Chang, F.-J., Barbosa-Cánovas, G.V., and Swanson, B.G., 1996. Inactivation of *Escherichia coli* by combining pH, ionic strength and pulsed electric field hurdles. *Food Res. Int.* 29: 117–121.
- Versari, A., Biesenbruch S., Barbanti, D., Farnell, P.J., and Galassi, S., 1997. Effect of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices. *Food Res. Int.* 30: 811–817.
- Wang, Z., Ma, Y., Zhao, G., Liao, X., Chen, F., Wu, J., Chen, J., and Hu, X., 2006. Influence of gamma irradiation on enzyme, microorganism, and flavour of cantaloupe (*Cucumis melo* L.) juice. *J. Food Sci.* 71: M215–M220.
- WHO/FAO, 2003. Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series p. 916. Geneva, Switzerland.
- Will, F., Bauckhage, K., and Dietrich, H., 2000. Apple pomace liquefaction with pectinases and cellulases: Analytical data of the corresponding juices. *Eur. Food Res. Technol.* 211: 291–297.
- Wouters, P.C., Alvarez, I., and Raso, J., 2001a. Critical factors determining inactivation kinetics by pulsed electric field food processing. *Trends Food Sci. Technol.* 12: 112–121.
- Wouters, P.C., Bos, A.P., and Ueckert, J., 2001b. Membrane permeabilization in relation to inactivation kinetics of *Lactobacillus* species due to pulsed electric fields. *Appl. Environ. Microbiol.* 67: 3092–3101.
- Wouters, P.C., Dutreux, N., Smelt, J.P.M., and Lelieveld, H.L.M., 1999. Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Appl. Environ. Microbiol.* 65: 5364–5371.
- Yen, G.-C. and Lin, H.-S., 1996. Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf-life of guava puree. *Int. J. Food Sci. Technol.* 31: 205–213.
- Yeom, H.W., Streaker, C.B., Zhang, Q.H., and Min, D.B., 2000. Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *J. Agric. Food Chem.* 48: 4597–4605.
- Yuan, Y., Hu, Y., Yue, T., Chen, T., and Lo, Y.M., 2009. Effects of ultrasonic treatments on thermoacidophilic *Alicyclobacillus acidoterrestris* in apple juice. *J. Food Process. Preserv.* 33: 370–383.
- Zhang, G., Ji, B., Li, B., Tian, F., Chen, G., Ji, F., Zhang, H., Yang, Z., and Zhao, L., 2008. Effect of processing and storage condition on phenolic concentration and antioxidant activities of apple and apple juices. *J. Food Sci. Technol., Mysore* 45: 339–343.
- Zhang, Q., Monsalve-González, A., Qin, B.L., Barbosa-Cánovas, G.V., and Swanson, B.G., 1994. Inactivation of *Saccharomyces cerevisiae* in apple juice by square-wave and exponential-decay pulsed electric fields. *J. Food Process Eng.* 17: 469–478.


Q11

Q12,Q13

**TO: CORRESPONDING AUTHOR****AUTHOR QUERIES - TO BE ANSWERED BY THE AUTHOR**

The following queries have arisen during the typesetting of your manuscript.  
Please answer these queries by marking the required corrections  
at the appropriate point in the text.

Q1	Please confirm whether the change from Diplock et al., 1998 to Diplock et al., 2003 as per reference list is OK.	
Q2	Please confirm whether “quercetin glycosides” or “quercetin glycosides”.	
Q3	Please confirm the abbreviation given for “United States Food and Drug Administration”.	
Q4	Please provide expansion for “RDA”.	
Q5	Please provide expansion for “HPLC”.	
Q6	Please confirm whether the change from Dogan and Erkmen, 2004 to Dogen and Erkmen, 2004 as per reference list is OK.	
Q7	Please confirm whether the change from Garcia et al., 2001 to García et al., 2001 as per reference list is OK.	
Q8	Please provide expansion for “HPP”.	
Q9	Please confirm whether the change from Spilimibergo et al., 2007 to Spilimbergo et al., 2007 as per reference list is OK.	
Q10	Please confirm the change from “Pom Wonderful” to “POM Wonderful”.	

Q11	Please update the reference Stevenson and Lowe (2009) with volume number and page range.	
Q12	Please check and confirm whether the usage of “ <i>Mysore</i> ” in journal title of Zhang et al. (2008) is OK.	
Q13	Reference Zhang et al. (2008) is not cited in the text. Please cite at an appropriate place(s).	