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Pome Fruit Juices

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1.1 Introduction

Apple and pear are the two major commercial importance pome fruits that are grown in most temperate regions of the world. Apples (\textit{Malus domestica}) have a strong antioxidant activity, which is mainly being attributed to the polyphenolic fraction. Pears (European pears: \textit{Pyrus communis} L.; Asian pears: \textit{Pyrus serotina} L.) are a good source of dietary fiber and vitamin C. Both apples and pears are often consumed fresh and also canned, dried, baked, freeze-dried, and as a cloudy or concentrate juice. The concentrated pome juices are usually obtained by extraction or pressing and later, clarification. The first step produces a juice of about 12\textdegree Brix, and, after concentration, a final product of about 70\textdegree Brix is obtained (Falguera \textit{et al.}, 2013). However, its properties are also constantly changing when the juices are subjected to processing, storage, transport, marketing, and consumption (Rao, 1986). Through the years, the process of optimization for obtaining and preserve these products has been conducted in order to avoid undesirable quality changes. This chapter discusses the application of conventional and emerging techniques in the processing of pome fruit juices and their effect on the final quality of the product.

1.2 Conventional Processing Techniques

The juice production starts with handling, which, in the case of apples and pears, is with the use of water or conveyor belts. Rotten or moldy apples and pears should be removed. Later, washing is required to remove leaves and twigs and dirt or other

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water-soluble agricultural spray residues. Sanitizing process will then be carried out to avoid a high microbial load, undesirable flavors, or mycotoxin contamination (Barret et al., 2005).

Prior to juice extraction, the fruit has to be pulped to release the trapped juices, and in the case of apples and pears, they need to be pressed at fairly high pressure to force the juice to flow through the cell structure (Downes, 1995). The fruits are milled to a pulp by a disintegration process that starts with a crushing step to break down the cell tissue. When fruits have been crushed, it undergoes a pressing step where the juice is extracted from the fruit by using conventional pack presses or horizontal rotatory presses. Juice extraction can also be performed by pectolytic enzymes, but apples and pears can normally be pressed fresh without any assistance. In general, juice-extraction process should be carried out as quickly as possible in order to minimize oxidation of the product. When juice has been extracted, clarification and filtration methods are generally conducted depending on the characteristics of the final product. For cloudy juices, clarification will not be necessary and only controlled centrifugation or a coarse filtration will be conducted to remove larger insoluble particles. To obtain a clarified juice, it will be necessary to remove the turbidity by a clarification step. Therefore, a complete depectinization by addition of pectinase enzymes, fine filtration, or high-speed centrifugation will be required (Barret et al., 2005). To obtain concentrate juices, a multiple-stage evaporation process after clarification is carried out. The clarified juice has a soluble solid content of around 15°Brix, and processing industries normally obtain juices with a content of 70°Brix. Hence, in the concentration process, the juice changes its soluble solids content and temperature flows from one evaporator. This process, which often works at a temperature of 60°C, not only preconcentrates the juice but also subjects the product to some reduction in quality and loss of nutrients (Falguera & Ibarz, 2014). Then, the concentrate is immediately cooled below 20°C (NPCS, 2008). In order to destroy microorganisms and inactivate the enzymes that are present in all the obtained products, thermal pasteurization process is conducted in a flow-through heat exchanger by high-temperature–short-time (HTST) treatment, for example, 76.6–87.7°C for holding time between 25 and 30 s (Moyer & Aitken, 1980). In-pack pasteurization directly processes into the preheated pack ensuring product integrity (e.g., held at 75°C for 30 min) (NPCS, 2008). However, slight correction of acidity of pome fruit juices to reach equilibrium pH of 4.6 or below by addition of commercial citric acid is necessary before pasteurization process. Pome fruit juices can also be processed by an aseptic method, in which temperatures are risen well over 100°C, but holding times are shortened to only few seconds. Aseptic technology, also known as ultrahigh-temperature (UHT) processing, involves the production of a sterile product by rapid heating at high temperatures, followed by a short holding time and ending with rapid cooling (Charles-Rodríguez, 2002).

1.2.1 Influence on Microbial Quality

Microflora present in fruit juices are normally associated to the surface of fruits during harvest and postharvest processing including transport, storage, and processing (Tournas et al., 2006). The severity and extent of the thermal treatment will depend on factors such as type and heat resistance of target microorganisms, spore or enzyme present in food, pH, oxidation–reduction potential, or water activity of the food (Ramaswamy & Singh, 1997). The pH is also a critical factor, taking into account that
foods may be broadly divided into high-acid (pH < 3.7) and low-acid (pH > 4.5) foods. Apple juices have the pH range between 3.35 and 4.00 serving as important barrier for microbial growth. Pears are not abundant in ascorbic acid (Kopera & Mitek, 2006) and some pear varieties have pH sometimes higher than 4.6 (Visser et al., 1968). The acidic nature of apple or pear juices allows pasteurization, defining the use of temperatures close to 100 °C in order to inactivate spoilage microorganisms. However, when pH is greater than 4.6, spore heat resistance marks the process temperature that should be greater than 115 °C for extended shelf life. Therefore, the pH reduction by acid addition is a widely practice in the juice food industries.

Spoilage organisms of particular interest to fruit juice manufacturers are Alicyclobacillus and yeasts and molds. Alicyclobacillus acidoterrestris is acidophilic spore-forming, heat-resistant organism that may be found in fruit juices. This microorganism can survive commercial pasteurization processes commonly applied to apple and pear juices spoiling fruit juice by producing cloud loss, development of off-flavors, CO₂ production, or changes in color and appearance (Lawlor et al., 2009). Saritas et al. (2011) evaluated the effect of ascorbic and cinnamic acid addition and different pasteurization parameters on the inactivation of A. acidoterrestris. They concluded that the increase in the acid concentrations led to a decline in the z-value of the microorganisms, which is greater in the clear apple juice than in the apple nectar.

Several studies have been conducting regarding the recommended pasteurization treatments in apple juice. Mazzotta (2001) recommended a general thermal process of 3 s at 71.1 °C for achieving a 5-log reduction of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in apple juices adjusted to a pH of 3.9. They reported that pH ranges from 3.6 to 4.0 in apple juice had no significant impact on the heat resistance of E. coli O157:H7. However, the acid tolerance of some microorganisms including E. coli and Salmonella led to the use of chemical preservatives such as benzoic acid in pome fruit juices to help processors to increase, substantially, the safety of the pasteurized products (Albashan, 2009; Koodie & Dhople, 2001). According to the Food and Drug Administration (FDA) recommendations, the pasteurization process must ensure a 5-log reduction of the three stated vegetative bacterial pathogens (E. coli O157:H7, Salmonella, and L. monocytogenes) at pH values of 3.9. However, thermal destruction of the protozoan parasite Cryptosporidium parvum oocysts causing illness outbreaks associated with the consumption of apple juice must be taken into consideration. Published studies suggest that C. parvum might be more resistant to heat processing than the indicated three vegetative bacterial pathogens (Deng & Cliver, 2001). Therefore, FDA suggests a treatment at 71.7 °C for 15 s for apple juice at pH values of 4.0 or less to achieve the 5-log reduction for the three mentioned vegetative bacterial pathogens and for oocysts of C. parvum (FDA, 2004). For the case of pear juice with pH of 4.6 or less, FDA recommends to conduct the pasteurization process that guarantees the same 5-log reduction for the indicated microorganisms. Regarding patulin, a mycotoxin produced by certain species of Penicillium, Aspergillus, and Byssochlamys molds, the regulations limit their content in apple juice to no more than 50 μg/kg (Codex, 1999).

1.2.2 Influence on Nutritional Attributes

Apple and pear cultivars are well known for being good sources of antioxidant properties, especially for their content of polyphenolic compounds concentrated in their
Apples contain natural sugars, organic acids, dietary fiber, minerals, and vitamins, whereas pears are good sources of fiber and micronutrients such as chlorogenic acid, flavonols, and arbutin (Sinha, 2012). In general, pear juice is characterized by similar soluble solids content than apple juices, but in sugar-free extract, sorbitol content has higher contribution, which is estimated to be 10–25 g/l (Dietrich et al., 2007).

Thermal processing inactivates spoilage microorganisms efficiently but may also degrade nutritional quality of foods (Qin et al., 1995). According to Charles-Rodríguez (2002), conventional UHT treatment has apparent effects on pH, soluble solids, and acidity of the processed apple juice. Throughout the years, process optimization has been carried out in order to reduce processing times at high temperatures and avoid undesirable quality changes during the process (Awuah et al., 2007). Nevertheless, thermal processing can still induce reactions that could affect color, odor, flavor, texture, and health-related compounds, which all could be linked to the change in phenolic profile (Niu et al., 2010). Aguilar-Rosas et al. (2007) reported that HTST treatment caused a considerable loss of phenols (32.2%) in apple juices. These results agree with those reported by Spanos and Wrolstad (1992) who reported that total phenol concentration is reduced up to 50% in thermally treated apple juice at 80 °C for 15 min. Gardner et al. (2000) also observed a considerable loss of phenolics in pasteurized apple juice by thermal means. De Paepe et al. (2014) identified 42 compounds to be susceptible to thermal degradation, indicating that the most heat labile phenolic constituent in cloud apple juice was procyanidin subclass, according to the high degree of structural elucidation achieved. Jiang et al. (2016a) also observed an important decrease of 53.11% and 46.47% in the total phenolic and flavonoid content of pear juice concentrates from Hwasan and Niitaka cultivars. They indicated that the antioxidant levels were significantly affected during the pear juice production, especially during the pressing step in which press cake waste retains the seed and skin. A complete inactivation of enzymes (peroxidase, POD; pectin methylesterase, PME; and polyphenol oxidase, PPO) and microorganisms (total plate count, yeasts, and molds) was observed in conventional pasteurization at 95 °C, but this treatment also showed highest losses of ascorbic acid, total phenols, flavonoids, and antioxidant capacity (Saeeduddin et al., 2015).

Moreover, during the clarification step of juice processing, a part of polyphenols is also lost (Dietrich, 2004; Hubert et al., 2007). In contrast, the enzymatic and clarification stages are avoided in cloudy juices, which results in higher phenolic content in the final product. This means that cloudy juices have higher antioxidant activity and dietary fiber than clear ones (Oszmianski et al., 2007). Willems and Low (2016) studied the effect of enzyme treatment and processing on the oligosaccharide profile of commercial pear juice samples. They observed that the majority of polysaccharide hydrolysis and oligosaccharide formation occurred during the enzymatic treatment at the pear-mashing stage, and the remaining processing steps had a minimal impact on the carbohydrate-base chromatographic profile of pear juice.

A reduction in nutritional quality could also be caused by browning reactions of fruits during processing and storage. Due to the general low acidity of pears, the addition of antibrowning agents to pear juice can lower the pH with a suitable sugar–acid ratio. According to Jiang et al. (2016b), addition of 0.20% ascorbic acid might solve the browning problem as well as improve the antioxidant capacity of pear juice.
1.2.3 Influence on Organoleptic Attributes

Thermal pasteurization of apple and pear juices is effective in preventing microbial spoilage and extends their shelf life. However, these treatments induce a negative impact on their natural flavor and color that is perceived by consumers (Mak et al., 2001).

1.2.3.1 Changes in Aroma Profile  Volatile composition and content in apple and pears is strongly influenced by the variety, maturity, and storage (Sinha, 2012). Different studies reported that ester compounds like ethyl butyrate, ethyl-2-methyl butyrate, butyl acetate, and hexyl acetate are key odorants giving the “apple” flavor; hexyl acetate the “sweet-fruit,” and 1-butanol the “sweetish sensation” (Lavilla et al., 1999; Mehinagic et al., 2006; Sinha, 2012). Regarding pear aroma, compounds including hexanal, cinnamaldehyde, methyl and ethyl decadienoates, and farnesenes have been reported to be responsible for apple aroma (Riu-Aumatell et al., 2005). Few studies have been focused on the volatile composition of pasteurized pome fruit juices. It is generally accepted that a conventional thermal juice pasteurization treatment induces a significant loss of the fresh flavor content (Braddock, 1999). In apple juice, a decrease in the aroma components including iso-butyl acetate, ethyl butyrate, ethyl 2-methyl butyrate, and hexanal was observed after pasteurization at 85°C for 10 min (Su & Wiley, 1998). An HTST treatment at 90°C for 30 s resulted in more than 50% loss in hexanal, ethyl acetate, ethyl butyrate, methyl butyrate, and acetic acid concentrations in apple juice (Aguilar-Rosas et al., 2007). Kato et al. (2003) concluded that the odor attributes of pasteurized apple juices could not be predictable. However, they could determine that treatment temperature affected the apple juice volatile concentration more than the treatment time.

The effect of heat on apple and pear juice aromas could be stated during the juice concentration process. Volatiles are normally recovered and concentrated for later addition of the concentrated products, resulting in the loss of volatiles if incomplete restoration takes place (Renard & Maingonnat, 2012). In apple juices, conventional evaporation could induce a loss of more than 95% of trans-2-hexenal (Onsekizoglu et al., 2010). Steinhaus et al. (2006) observed a distinct loss of the major juice aroma component β-damascenone during apple juice concentration, and high losses were also found for dimethyl sulfide and 1-octen-3-one.

1.2.3.2 Changes in Appearance (Color and Juice Clarity)  Cloudiness and color stability are two of the main important quality characteristics for cloudy apple and pear juices, strongly influenced by the enzyme activities of pectinesterase (PE) and PPO. In general, HTST treatment can promote the inactivation of these enzymes but their action begins from initial processing steps such as the breakage of the fruit during the juice-extraction process (Krapfenbauer et al., 2006). In addition to heat treatments, other methods including blanching, pectolytic enzyme treatments, or the use of antibrowning agents are some of the strategies to control browning and cloud destabilization (Fukutami et al., 1986; Gierschener & Baumann, 1988; Castaldo & Loidice, 1997; Quoc et al., 2000; Riahi & Ramaswamy, 2003). Krapfenbauer et al. (2006) focused their study on the impact of HTST treatment combinations (60–90°C...
for 20–100 s) on enzymatic browning and cloud stability of different apple juices considering eight apple varieties. They concluded that HTST at 80 °C inactivated PPO and reduced PE activity at 50%. In addition, they indicated that the best stability of cloud and color in relation to heat treatment was observed at 70 °C/100 s and 80 °C/20 s.

Sun (2012) reported that the influence of HTST treatment at 73, 80, or 83 °C for 27 s on apple juice induced values of total color difference (TCD) of 1.3. TCD reflects the overall color difference evaluation between the reference sample, normally that of initial fresh product, and the final processed product. The accumulation of brown color during thermal treatment of juices is attributed to nonenzymatic reactions involving caramelization and Maillard reactions (Ibarz et al., 1990). Nonenzymatic browning kinetics of concentrated pear juices from three pear varieties (“Alexandrine Douillard,” “Flor de Invierno,” and “Blanquilla”) has been studied at three temperatures (90, 80, and 70 °C) and different soluble solid contents (52, 62, and 72 °C) (Ibarz et al., 1997). The “Alexandrine Douillard” pear juices showed less nonenzymatic browning in contrast to the “Flor de Invierno” and “Blanquilla” juices, but formation of 5-hydroxymethyl-2-furfuraldehyde followed first-order kinetics in all products. Burdurlu and Karadeniz (2003) evaluated the kinetics of nonenzymatic browning in Golden Delicious and “Amasya” apple juice concentrates for 4 months. They reported an increase in browning level of all apple juices according to a zero-order reaction kinetics. Hsu et al. (1990) observed higher degree of browning in juice and concentrate from “Barlett” than “Comice” and “Anjou” pears, which attributed to the high amino acid content.

1.3 Novel Processing Techniques

The application of promising novel processing techniques in the manufacture of pome fruit juices is being established in different steps of their production chain. On the other hand, the production process of pome fruit juices includes the steps of extraction and clarification. The yield of juice extracted by the application of pretreatments has been explored to ensure the highest possible quality of the product and increase the juice recovery. Clarification process allows to obtain a final product without turbidity and to preserve its organoleptic properties. Novel clarification processes have some advantages in relation to the conventional processes taking into account the final sensory and nutritional properties of the product. Moreover, novel food preservation approaches including physical methods based on nonthermal treatments, chemical methods such as natural food preservatives, and their combinations for extending their shelf life and also preserving natural organoleptic and nutritional value are result-oriented novel techniques currently applied in these products.

1.3.1 Improvement in Juice Extraction

Juice extraction is the process where the liquid phase is separated from the solid particles (Girard & Sinha, 2012). Juice extraction can be carried out using various mechanical processes, which may be achieved through diffusion extraction, decanter centrifuge, screw type juice extractor, fruit pulper, and by different types of presses (Sharma et al., 2016). Crushing operation prior to pressing can be divided into
chopping and preparation for pressing. In addition, enzymatic treatment prior to mechanical extraction significantly improves juice recovery compared to any other extraction process (Sharma et al., 2016). In this way, pectinase and polygalacturonase (PG) enzymes, which act on glycosidic linkage acting as fruit softening, can be used as a press aid adding to milled/crushed apples before the juice-extraction step (Sinha, 2012).

According to Bazhal and Vorobiev (2000), electrotechnologies such as electro-osmotic (EOs) treatments and pulsed electric fields (PEFs) could intensify the juice-extraction yield. EO treatments lead to addition or extraction of liquid from capillary-porous materials. The application of PEF as a pretreatment operation before pressing could allow significant increase in the juice yield and obtaining products with higher quality (Vorobiev et al., 2004). Bazhal and Vorobiev (2000) carried out an experiment with Golden Delicious apple slices in a laboratory filter-press cell fitted with an appropriate electric treatment system. PEF treatments consisted of 1000 monopolar pulses of 1000 V with duration of 100 μs and a period of 10 ms, whereas EO treatments consisted of successively 50, 100, and 200 mA for 30 min each. They observed that both EO and PEF treatments caused significant increase in apple juice yield. However, the energy consumption was 50–100 times less for PEF treatments. Wu and Zhang (2015) evaluated the influence of PEF pretreatment on apple tissue structure, confirming an evident disorder in cells of fresh apple caused by the electroporation and the membrane damage of PEF. However, the pretreated samples did not collapse and maintained a desirable structure. Carbonell-Capella et al. (2016) studied the impact of apple pretreatment by PEF on juice extraction using the freezing-assisted pressing. They observed that freezing-assisted pressing at subzero temperatures was an effective tool in order to obtain an apple juice rich in bioactive compounds. In addition, the process was improved by the application of PEF pretreatment of apple tissue before freezing resulting in a reduction of both freezing and thawing time, and pressing was more effective.

### 1.3.2 Improvement in Juice Clarification

At industrial level, clarification of pome fruit juices is normally conducted mechanically or enzymatically. Mechanical clarification normally uses centrifuges and filtration devices by the first filtration phase in settling centrifuges, meanwhile decanters are used to remove fibers from cloudy juices (Welter et al., 1991; Nagel, 1992). Conventional filtration methods are labor intensive and time consuming (Zhao et al., 2014). New filtration methods such as ultrafiltration (UF) require lower labor and energy operation cost and have lower waste disposal in comparison to conventional methods (de Bruijn et al., 2003; Zhao et al., 2014). UF has shown excellent quality attributes for clarified apple juice (Álvarez et al., 2000; De Bruijn et al., 2002). Álvarez et al. (2000) proposed an integrated membrane process for producing apple juice and apple juice aroma concentrates. The process involved an integrated membrane reactor to clarify the raw juice, reverse osmosis to preconcentrate the juice up to 25 °Brix, prevaporation to recover and concentrate the aroma compounds, and a final evaporation step to concentrate apple juice up to 72 °Brix. The obtained juice was more clear and brilliant than apple juice produced by conventional methods, and the system had more economical advantages than the conventional process. The combination of UF (0.05 μm) with ceramic membrane and high hydrostatic pressure processing (HHP) at 500 MPa
for 6 min to process fresh apple juice shows good results in terms of color, clarity, and total phenols retention, leading to microbiologically safe products for 60 days at 4 °C (Zhao et al., 2014).

Pectic enzymes, that is, PME and PG, are often used in combination with amylases and cellulases in fruit and vegetable juice clarifications to obtain higher juice yields and clarity (Raju & Bawa, 2012). Fungal PG is normally used in the industrial processes for juice clarification. However, recently clarification of juice has been carried out through recombinant and nonrecombinant fungal strains. Singh and Gupta (2004) evaluated the effect of gelatin on the efficacy of a fungal pectinolytic enzyme preparation from Aspergillus niger van Tieghem for clarification of apple juice. They observed a complete clarification of the apple juice (200ml) using 0.01% gelatin and 15 IU of enzyme preparation at 45 °C after a holding time of 6h. In addition, the scaling up to 200 times results in about 143% more transmittance than the control juice, and the clarified juice stored at room temperature did not show any haze development after 2 months.

1.3.3 Preservation of Pome Fruit Juices by Innovative Technologies

1.3.3.1 Pulsed Electric Field Processing Several researchers (Qin et al., 1994; Ortega-Rivas et al., 1998; Zárate-Rodríguez et al., 2000; Aguilar-Rosas et al., 2007) have successfully studied the application of PEF processing for extending the shelf life of apple juices. The application of high voltage induced structural changes in membranes of microbial cells based on electroporation, leading to microbial inactivation (Tsong, 1991; Barbosa-Cánovas et al., 1999). Energy savings for PEF processing have been reported in comparison to conventional thermal processing in addition to being more environmentally friendly (Toepfl et al., 2006). The PEF treatment has been reported to use less than 10% of the electric energy for heat treatment of apple juice (Qin et al., 1994). Aguilar-Rosas et al. (2007) observed that PEF treatment at 35 kV/cm and a frequency of 1200 pulses per second in bipolar mode using pulses of 4 μs wide pasteurized an apple juice from Golden delicious fruits. They reported that PEF treatment proved to be efficient in microbial inactivation and induced better preservation in quality attributes than a conventional HTST pasteurization. Ortega-Rivas et al. (1998) compared UF and PEF in apple juice pasteurization reporting 6-log reductions in survivability of total aerobic microorganisms using the indigenous flora of the PEF-treated product with no differences in quality. Evrendilek et al. (2000) studied the microbial safety and shelf life of PEF-treated apple juice showing that PEF (35 kV/cm for 94 μs total treatment time) not only could extend the shelf life of the fresh product but also maintained a fresh flavor. They reported an inactivation of E. coli O157:H7 of 4.5-log by the PEF treatment and no changes in the natural food color and vitamin C of the product. Timmermans et al. (2014) conducted experiments using a continuous-flow PEF system at 20 kV/cm with variable frequencies to study the inactivation of Salmonella panama, E. coli, L. monocytogenes, and Saccharomyces cerevisiae in apple juice. They observed that under the same conditions, S. cerevisiae was the most sensitive microorganism, followed by S. panama and E. coli. A synergistic effect between the inlet temperatures above 35 °C and PEF treatment was demonstrated, suggesting that optimization of the PEF conditions to
reduce the energy input should aim for processing at higher inlet temperature to allow more effective inactivation per pulse. Applying PEF treatment at 30.76 kV/cm and using up to 21 pulses in apple juice achieved the 5-log reduction of *E. coli*, FDA-required standard for alternative pasteurization methods (Moody *et al*., 2014). Little information is available regarding the effect of PEF on pear juices. Jin *et al*. (2008) evaluated the application of PEF at 30 kV/cm for 240 μs at 200 Hz and 10 °C on the microbial inactivity and quality of freshly squeezed pear juice. They reported an inactivation of *E. coli* and *S. cerevisiae* of 4.6 and 2.7-log after the PEF treatment, with no effect on the physicochemical properties and nutrition of the product.

Related to enzyme inactivation, Giner *et al*. (2001) reported a decrease in PPO activity up to 3.15% at 24.6 kV/cm for 6 ms of treatment time, whereas a total inactivation of 38% was obtained for pear PPO at 22.3 kV/cm for the same treatment time. Almost a complete inactivation of PPO and POD was achieved after treating apple juice at 35 kV/cm and 2 ms of pulse rise time (Bi *et al*., 2013). The same PEF treatment conditions led to apple juices with significant higher lightness and yellowness than the control samples as well as preservation of initial total phenol content. No differences between PEF-treated apple juices samples (1, 3, 5 kV/cm, n = 30 pulses) and fresh samples were reported related to the overall apple juice composition described by pH, total soluble solids, total acidity, density, contents of sugar, malic acid, and pectin as well as polyphenol contents and antioxidant capacity (Schilling *et al*., 2007). Charles-Rodríguez *et al*. (2007) also reported that PEF treatment seemed to retain more the color of natural apple juice than thermally pasteurized juices since less browning effect was observed. Harrison *et al*. (2001) reported that PEF-treated apple juice could be stored at 4 °C for 1 month with no change in the volatile flavor profile.

Combination of preheating at 40 °C and PEF treatment has been shown to induce an antimicrobial effect while avoiding detrimental effects on the quality of the product and inactivated PPO enzymes in apple juice (Heinz *et al*., 2003; Ertugay *et al*., 2013). Riener *et al*. (2008) reported that preheating at 50 °C and PEF treatment at 40 kV/cm for 100 μs achieved a PPO inactivation of 71% in PPO apple juice. Similar conditions combining preheating at 50 °C and PEF processing at 38.5 kV/cm and 300 pulses per second reduced a 70% of PPO activity in apple juice (Sanchez-Vega *et al*., 2009). Synergistic lethal effects of PEF treatment and carvacrol have been reported in relation to increase the microbial inactivation in apple juices (Ait-Ouazzou *et al*., 2013). PEF treatments consisting of 50 exponential decay pulses at 30 kV/cm induced less than 1-log cell cycle reduction, while the combination with 1.3 mM of carvacrol caused the inactivation of 5-log cell cycles with 20 pulses. Similar synergistic lethal effects on apple juice have been observed for the combination of essential oils including *Cyperus longus* L., *Eucalyptus globulus* L., *Juniperus phoenicea* L., *Mentha pulegium* L., *Rosmarinus officinalis* L., and *Thymus algeriensis* and mild PEF treatment (30 kV/cm, 25 pulses) (Ait-Ouazzou *et al*., 2012). Mosqueda-Melgar *et al*. (2008) also reported that combinations of PEF treatment (35 kV/cm, 4 μs, <40 °C) with 0.1% cinnamon bark oil or 1.5% citric acid achieved more than 5-log reductions in microbial populations and extended the shelf life of apple and pear juices for 91 days at 5 °C.

The application of PEF and membrane UF as nonthermal preservation technique to process apple juice has been shown to be very efficient in preserving the quality attributes in terms of soluble solids, pH, acidity, and color ratio of the product (Zárate-Rodríguez *et al*., 2000). Noci *et al*. (2008) studied the influence of ultraviolet irradiation (UV) and PEF on microbial inactivation, quality attributes, and enzymatic activity of fresh apple juice. They observed that application in combination UV + PEF
or PEF + UV achieved similar reductions of 6.2- and 71-log cycles, respectively, of total bacterial counts. Both combinations also showed better retention of juice color and level of phenolic compounds as well as better reduction of PPO and POD activities in comparison to heat pasteurization. Walkling-Ribeiro et al. (2008) reported a higher reduction of S. aureus with a hurdle approach (UV; 46 °C (PEF inlet) and 58 °C (PEF outlet); PEF 40 kV/cm and 100 μs) in comparison to conventional pasteurization (9.5- vs 8.2-log, respectively) with little effect on the quality of apple juice.

1.3.3.2 High Hydrostatic Pressure Processing HHP processing has been proved to meet the FDA requirement of a 5-log reduction of microorganisms in pome fruit juices without changes in the sensory and nutritional characteristics of the product due to the low processing temperature. Ramaswamy et al. (2003) achieved more than 5-log reduction of E. coli 29,055 after treatment of 400 MPa at 25 °C. Moody et al. (2014) also achieved 7-log reduction of E. coli after treatment of 400 MPa for 3 min. Evelyn et al. (2016) evaluated the efficacy of HHP at 600 MPa in combination with 75 °C to inactivate Neosartorya fischeri, a mold that spoils apple juice and can produce mycotoxins. They observed around 3.3-log reduction after the HHP-75 °C treatment for 10 min, suggesting that HHP could be an option for apple juice preservation. According to Juarez-Enriquez et al. (2015), HHP treatment consisting of 430 MPa for 7 min could extend shelf life of apple juice for 34 days with no changes in the physicochemical properties, nutritive value, or sensory attributes. In addition, PME and PPO PPO activities were controlled by the HHP treatment.

Combination of ultrafiltration (UF, 0.05 μm) and HHP treatments (500 MPa for 6 min) showed that total plate count and yeasts and molds decreased below 1-log cycle after the treatment of apple juice (Zhao et al., 2014). In addition, the product showed lower browning degree, higher total phenols and clarity, and better retention of main volatile aroma compounds than HTST for 60 days of storage at 4 °C. The combination of HHP processing with nisin for the inactivation of A. acidoterrestris spores in apple has also been evaluated. HHP treatment of 200 MPa for 45 min with a nisin content of 250 IU/ml enabled total spore inactivation (Sokolowska et al., 2012).

1.3.3.3 Other Innovative Technologies for Preservation Purposes The application of other innovative physical methods has been explored to preserve pome fruit juices including ultrasound (US), dense phase carbon dioxide (DPCD) processing, ultraviolet light-C (UV-C), pulsed light (PL), high-pressure homogenization (HPH), or ohmic heating (OH).

The application of US has showed positive results for apple juice pasteurization meeting the criteria of the FDA, concerning a 5-log reduction of microbial cells in the fruit juices. Yuan et al. (2009) reported a 80% of reduction of A. acidoterrestris when treating apple juice at 24 kHz, 300 W for 60 min. Moody et al. (2014) also reported more than 6-log reduction after 5 min of US treatment at 400 W and 24 kHz, keeping the temperature at 60 °C. Bastianello et al. (2016) investigated the microbial shelf life and the volatile compounds of cloudy apple juices after US treatment (400 W and 24 kHz at 35 °C applying a process of 360 s/100 ml). They observed a sublethal injury of spoiler yeasts (Candida parapsilosis and Rhodotorula glutinis) 14 days post treatments, estimating the shelf life of the product to be around 21 days. The typical aroma compounds of untreated samples decreased in comparison to the US-treated juices. US treatment has shown to have some effect on PPO inactivation in apple juice at 23 °C and applying US power densities of 3300 W/l for 20 min (Silva et al.,
In 2015, Sun et al. (2015) also observed that US inhibited the browning of fresh apple (*Malus pumila Mill*, cv. *Red Fuji*) juice by applying an US intensity of 2 W/cm² at 15 °C for 10 min but decreased the total phenolic content and antioxidant capacity of the product. Abid et al. (2014) also observed that US treatment (20 kHz, 20 °C for 3 min) did not change the total soluble solids, pH, and acidity of apple juice, while significant increase in nonenzymatic browning and cloud values was observed. Ertugay and Başlar (2014) also observed that US treatment increased the cloudiness level up to 16.9 times and the cloud stability up to 9.8 times of apple juice. Saeeduddin et al. (2016) reported that US treatment at 25 kHz for 60 min at 25 °C exhibited optimum results in terms of physicochemical and microbial quality of pear juice. Saeeduddin et al. (2015) also observed that US-treated (20 kHz at 10 °C for 65 °C) pear juice quality was not affected by the US treatment, but an improvement of phenolic compounds and ascorbic acid was achieved in the US-treated product. Synergistic effects of US (30 min at 20 kHz) and continuous flow-through PL system (0.73 J/cm², 155 ml/min) for the treatment of apple juice were observed by Ferrario and Guerrero (2016). They reported that the combined treatment delayed yeast and mold recovery and prevented browning development during the storage of the product. The combination of the US and mild temperatures, called thermosonication, has been attempted as an alternative thermal treatment for the processing of apple juices. Abid et al. (2014) observed a quality enhancement of apple juice in terms of enzyme and microbial inactivation when using ultrasound with-probe sonicator (20 kHz for 10 min at 60 °C).

Other nonthermal methods include the DPCD, based on a cold pasteurization in which the product is in contact with either (pressurized below 50 MPa) sub- or supercritical CO₂ for certain time (Porto et al., 2010). Only one study has been carried out in apple juice resulting in a pasteurization effect, minimizing the quality loss in terms of nutritional value and volatile compounds applying 15 MPa at 35 °C for 15 min or 25 MPa at 35 °C for 15 min (Porto et al., 2010).

The UV-C (200–800 nm) has been used as nonthermal method to successfully reduce the microbial load of apple juice. The good UV-C penetration in clear apple juice treated with UV dose of 1377 J/l showed a 7.42-log reduction of inoculated *E. coli* with no color changes (Keyser et al., 2008). Char et al. (2010) observed that UV-C treatments in a UV-C device, consisting of a 90-cm long UV-C lamp (100 W) placed inside a glass tube leaving an annular flow space (0.2 l/min, 40 °C), effectively inactivated microbial populations (*E. coli* and *S. cerevisiae*) in apple juice.

PL technology involves applying intense and short pulses of white light that have antimicrobial action due to the presence of a UV component within the broad spectrum of light flash (Maftei et al., 2014). Ferrario et al. (2015) reported that PL treatments of 3 pulses per second and fluencies ranging 2.4–71.6 J/cm² induced 3.8-log reductions in clear apple juice. According to Caminiti et al. (2012), treatments above 2.66 J/cm² achieved reduction levels below 1-log CFU/ml for both *E. coli* and *L. innocua* in apple juice. Treating with doses up to 10.62 J/cm² were in the same range as fresh samples in relation to the sensory evaluation of the products. Microbial reductions of 4-log-cycles for *E. coli* and 2.98-log-cycles for *L. innocua* were observed after applying PL fluences of 4 J/cm² in apple juices (Pataro et al., 2011). Reductions up to 3.76-log CFU/ml of *Penicillium expansum* were reported after applying 0.4 J/cm² per pulse, 40 flashes, and a depth of the juice of 6 mm with some darkening color changes (Maftei et al., 2014). Funes et al. (2013) suggested that PL treatments would be a potential alternative method to reduce patulin contamination in apple products including juices, since a significant decrease in patulin levels (up to 22%)
Table 1.1  Application of antimicrobial chemical treatments in pome fruit juices

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Storage conditions</th>
<th>Quality changes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple juice</td>
<td>Bacteriocin (brevicin from <em>Lactobacillus brevis</em> NS01)</td>
<td></td>
<td>Increase shelf life and clarification comparing to chemical agents (potassium sorbate or sodium benzoate)</td>
<td>Duraisamy et al. (2015)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>4.0 mM Sorbic acid, 1.5 mM cinnamic acid, 20 mM vanillin, 10 mM ferulic acid or 12 mM p-coumaric acid, 20 min at temperatures from 46 to 55 °C</td>
<td>4 °C</td>
<td>Complete inhibition of all initial yeast inoculum (10³ CFU/ml). Addition of antimicrobial prevented spoilage during storage</td>
<td>Wang et al. (2016)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Rosemary commercial extracts V20 and V40 at 7.8 mg/ml and 3.9 mg/ml, respectively</td>
<td></td>
<td>Reduce growth of alicyclobacilli vegetative cells but did not show sporidal effects on alicyclobacilli. Do not change sensory properties</td>
<td>Piskernik et al. (2016)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Propolis 0.1–0.2 mg/ml and heat treatment at 51 °C</td>
<td></td>
<td>Strong synergistic and lethal effects against <em>E. coli</em> O157:H7 Sakai and sensorially acceptable product</td>
<td>Luis-Villaroya et al. (2015)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Propolis extract at 1.2% and 5% concentrations</td>
<td></td>
<td>Antimicrobial activity against <em>E. coli</em> and <em>E. coli</em> O157:H7</td>
<td>Sagdic et al. (2007)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Grape seed extract (GSE) (0–1.9% v/v)</td>
<td>37 °C</td>
<td>Inhibition of growth of <em>A. acidoterrestris</em> cells and spore germination/outgrowth</td>
<td>Molva and Baysal (2015a)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Yerba mate extract 40 mg/ml</td>
<td></td>
<td>4.5-log reduction of <em>E. coli</em></td>
<td>Burris et al. (2012)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Chitosan 5 g/l</td>
<td>25 °C</td>
<td>Yeast-free conditions for 14 days</td>
<td>Roller and Covill (1999)</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>Temperature</td>
<td>Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------------------------------------------</td>
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<td>----------------------------------------------</td>
</tr>
<tr>
<td>Red apple juice</td>
<td>Six essential oils extracted from Shieh, kafoor and Neem (0.5%)</td>
<td>4 °C</td>
<td>Reduction of PPO. Increased the shelf life up to 4 weeks</td>
<td>Eissa et al. (2012)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Commercial pomegranate extract (POMELLA®, PE)</td>
<td>37 °C</td>
<td>Inhibition of growth of <em>A. acidoterrestris</em> cells and spore germination/outgrowth</td>
<td>Molva and Baysal (2015b)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Caprylic acid (0.6 mmol/l or 0.8 mmol/l) at 50 °C for 5 min</td>
<td>50 °C</td>
<td>Complete inactivation of inoculated <em>E. coli</em> (7.25–7.34-log CFU/ml)</td>
<td>Kim and Rhee (2015)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Carvacrol and <em>p</em>-cymene (0.25–1.25 mM) at 4 °C</td>
<td>4 °C</td>
<td>Undetectable levels of <em>E. coli</em> 0157:H7 for 19 days</td>
<td>Kiskó and Roller (2005)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>0.3% Cinnamon with 0.1% sodium benzoate</td>
<td>8 and 25 °C</td>
<td>Inactivation of 5.2-log CFU/ml of <em>E. coli</em> 0157:H7 in 11 days</td>
<td>Ceylan et al. (2004)</td>
</tr>
<tr>
<td>Apple juice (pH 3.3 and 3.8)</td>
<td>Vanillin (3000 ppm)</td>
<td></td>
<td>Reduction ranging from 4-log cycles after a 4–8 h exposure at 30 °C</td>
<td>Corte et al. (2004)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>2.5% (v/v) Malic acid</td>
<td>5 °C</td>
<td>Reduction of more than 5-log cycles of <em>L. monocytogenes</em>, <em>S. enteritidis</em> and <em>E. coli</em> 0157:H7 after 24 h inactivation</td>
<td>Raybaudi-Massilia et al. (2009)</td>
</tr>
<tr>
<td>Pear juice</td>
<td>2.5% (v/v) Malic acid</td>
<td>5 °C</td>
<td>Reduction of more than 5-log cycles of <em>L. monocytogenes</em>, <em>S. enteritidis</em> and <em>E. coli</em> 0157:H7 after 24 h inactivation</td>
<td>Raybaudi-Massilia et al. (2009)</td>
</tr>
<tr>
<td>Apple juice (pH 6.0)</td>
<td>Yerba mate extract 40 mg/ml</td>
<td>4.5-log</td>
<td>Reduction of <em>E. coli</em></td>
<td>Burris et al. (2012)</td>
</tr>
</tbody>
</table>
was observed after applying PL doses of 35.8 J/cm$^2$. In terms of nutritious quality, Orlowska et al. (2013) observed that PL treatments consisting of 31 J per pulse only reduced 1.30% vitamin C content.

On the other hand, HPH technology is considered to be a promising nonthermal technology, which involves combination of spatial pressure and velocity gradients, turbulence, impingement, cavitation, and viscous shear (Vasantha & Yu, 2012). Homogenization pressures from 100 to 200 MPa induced significant inactivation of *E. coli* K-12 in apple juice (Kumar et al., 2009). However, synergistic effects of the HPH treatment and chitosan at 0.1% concentration resulted in enhancing microbial inactivation. Pathanibul et al. (2009) reported more than 5-log reductions of *E. coli* and *L. innocua* when apple juice was exposed to homogenization pressures higher than 250 MPa. They observed interaction effects of 10 IU nisin in combination with the HPH treatment on *L. innocua*. Bevilacqua et al. (2012) observed a reduction by 2–4-log CFU/ml of *Saccharomyces bayanus* by applying homogenization pressures of 20 mPa in combination with 900 ppm of limonene and 2 ppm of citrus extract, respectively.

The potential of OH on the treatment of juices has been explored as an alternative method for sterilization or pasteurization. Jakób et al. (2010) observed that kinetic parameters related to inactivation of PME and POD in apple juice remained the same after OH compared to conventional indirect heating. Park and Kang (2013) studied the effect of electric field-induced (60 V/cm) OH for inactivation of *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *L. monocytogenes* in apple juice. They observed reduction between 3.40 and 3.59-log for all the three foodborne pathogens when processing the juice at 60 °C for 30 s. However, combinations at 58 and 60 °C with electric field increased the inactivation effect 2–3 times higher than those obtained after conventional thermal treatment. On the other hand, high lethal rate (6.39-log reduction) of *E. coli* K12 was obtained in apple juice when combining ultraviolet radiation and OH at 65 °C, leading to cell disruption and inhibition of cell replication (Lee et al., 2013).

The consumer demand for more natural products has led to finding natural methods for extending the shelf life of pome fruit juices. In this way, the application of natural antimicrobials including bacteriocins, lactoperoxidase, herbs, and spices containing essential oils or organic acids, also called GRAS (generally recognized as safe) substances, has shown feasibility for use in apple juice (Table 1.1).

Other alternative chemical methods such as the application of gaseous ozone (3 l/min flow rate and 2–3 g/m$^3$) treatments have been shown to be effective in the reduction of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* (Song et al., 2015).

### 1.4 Conclusion and Future Trends

The application of several novel processing technologies has been explored in particular in apple juices. However, the research in other pome fruit juices such as pear juices is still scarce. The application of nonthermal technologies is of particular interest in pome fruit juices since preservation of color and flavor characteristics in these products has been achieved. However, the interest for improving the quality characteristics and health-related compounds such as polyphenols in pome fruit juices is still a challenge. One approach would be to introduce new pome fruit cultivars and consider them as new varieties for juice making.
Acknowledgments

Dr Aguiló-Aguayo thanks the Spanish Government for the FPDI-2013-15583. L. Plaza thanks the National Institute for Agronomic Research (INIA) for a DOC-INIA research contract. Authors acknowledge financial support from CERCA Programme/Generalitat de Catalunya.

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