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Emerging Preservation Methods for Fruit Juices and Beverages

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1. Introduction

This chapter provide a review of traditional and non-traditional food preservation approaches including physical methods (non-thermal pasteurization), chemical methods (natural food preservatives) and their combinations for extension of the shelf life of fruit juices and beverages.

Traditionally, the shelf-life stability of juices has been achieved by thermal processing. Low temperature long time (LTLT) and high temperature short time (HTST) treatments are the most commonly used techniques for juice pasteurization. However, thermal pasteurization tends to reduce the product quality and freshness. Therefore, some non-thermal pasteurization methods have been proposed during the last couple of decades, including high hydrostatic pressure (HHP), high pressure homogenization (HPH), pulsed electric field (PEF), and ultrasound (US). These emerging techniques seem to have the potential to provide "fresh-like" and safe fruit juices with prolonged shelf-life. Some of these techniques have already been commercialized. Some are still in the research or pilot scale. The first part of the chapter will give an update of these emerging non-thermal techniques.

Apart from thermal pasteurization, some chemical preservatives are also widely used for the extension of the shelf-life of fruit juices and beverages. Two of the most commonly used preservatives are potassium sorbate and sodium benzoate. However, consumer demand for natural origin, safe and environmental friendly food preservatives has been increasing since 1990s. Natural antimicrobials such as bacteriocins, organic acids, essential oils and phenolic compounds have shown considerable promise for use in some food products. The second part of the chapter will comprise of applications of these natural antimicrobials in fruit juice preservation.

From scientific literature, it is apparent that some individual non-thermal methods as well as natural antimicrobials are effective to inactivate microorganisms or reduce the *log* colony forming units (CFU) while not adversely affecting the sensory and nutritional quality. Moreover, the combination of these techniques could also provide synergistic effects on prolonging the shelf-life of fruit juices and beverages and potentially could become replacements for traditional pasteurization methods. The third part of the chapter will provide recent progresses of these combined techniques in fruit juice shelf-life extension.

2. Traditional thermal pasteurization

Thermal processing is the most widely used technology for pasteurization of fruit juices and beverages. Juice pasteurization is based on a 5-*log* reduction of the most resistant microorganisms of public health significance (USFDA 2001). The process could be accomplished by different time-temperature combinations.

2.1 Low temperature long time (LTLT)

Fruit juice has been traditionally pasteurized by batch heating at 63-65°C for relatively long time (D'Amico et al. 2006). This method has been replaced by high temperature short time treatment due to the undesirable quality changes during this process.

2.2 High temperature short time (HTST)

HTST treatment could minimise those undesirable quality changes made by batch heating due to the much less duration of heat treatment. Currently, HTST pasteurization is the most commonly used method for heat treatment of fruit juice. For example, orange juice is processed by HTST at 90 to 95°C for 15 to 30 s (Braddock 1999). And apple juice is treated by HTST at 77 to 88°C for 25 to 30 s (Moyer & Aitken 1980).

3. Non-traditional method

Thermal processing has been proven to be effective for preservation of fruit juice and beverages. However, thermal treatment tends to reduce the product quality and freshness. Nowadays, consumer demand for natural, healthy and convenient food products is fast growing, which leads to the innovation of novel food preservation technologies. Based on the literature, these novel technologies can be generally divided into physical methods (mostly non-thermal methods) and chemical approaches.

3.1 Physical methods (Non-thermal pasteurization)

Some non-thermal pasteurization methods have been proposed during the last couple of decades, including high hydrostatic pressure (HHP), high pressure homogenization (HPH), pulsed electric field (PEF), and ultrasound (US). These emerging techniques seem to have the potential to provide "fresh-like" and safe fruit juices with prolonged shelf-life.

3.1.1 High hydrostatic pressure (HHP)

High hydrostatic pressure (HHP) processing uses pressures up to 1000 MPa, with or without heat, to inactivate harmful microorganisms in food products (Ramaswamy et al. 2005). High hydrostatic pressure has traditionally been used in non-food areas such as ceramic and steel production. The application of HHP in food area started from 1900s when Hite and other researchers applied HHP on the preservation of milk, fruits and vegetables. However, it takes a long time for the commercial products to emerge in the market. In 1990, the first HHP processed fruit jams were sold in the Japanese market. Subsequently, HHP processed commercial products including fruit juices and beverages, vegetable products, among others, have been produced in North America, Europe, Australia, and Asia (Balasubramaniam et al. 2008).

Generally, there are two **principles** that govern the behaviour of foods under pressure: the Le Chatelier-Braun principle and the Isostatic principle. The Le Chatelier-Braun principle indicates that any phenomenon (such as phase transition, change in molecular configuration, chemical reaction, etc.) accompanied by a decrease in volume is enhanced by the increase in applied pressure. The isostatic principle means that the distribution of pressure into the sample is uniform and instantaneous. Thus, the process time is independent of sample size and shape (Ramaswamy et al. 2005).

HPP is proven to meet the FDA requirement of a 5-*log* reduction of microorganisms in fruit juices and beverages without sacrificing the sensory and nutritional attributes of fresh fruits (San Martín et al. 2002). Compared with thermal processing, HHP has many **advantages**. It can provide safe product with reduced processing time. It can maintain maximum fresh-like flavor and taste in the product due to the lower processing temperatures. Moreover, it is environmentally friendly since it requires only electrical energy and no waste by-products generated (Ramaswamy et al. 2005, Toepfl et al. 2006). Due to these advantages, HHP has been widely used in food product preservation including fruit and beverages in the areas of microbial inactivation (Table 1) and shelf-life extension (Table 2).

Foods	Microorganism	Treatment parameters	*Log* reduction	Sources
Orange juice	*Escherichia coli* O157: H7	550 MPa, 30ºC, 5 min	6	Linton et al. 1999
Apple jam	*Listeria monocytogenes*	200 MPa, 20ºC, 5 min	2.8	Préstamo et al. 1999
Apple juice	*Escherichia coli* 29055	400 MPa, 25ºC	>5	Ramaswamy et al. 2003
Apple juice	*Escherichia coli, Listeria innocua, Salmonella*	545 MPa, 1min	5	Avure Technologies
Orange juice	*Escherichia coli, Listeria innocua*	241 MPa, 3 min	5	Guerrero-Beltran et al. 2011

Table 1. Examples of HHP inactivation of microorganisms in fruit products

Foods	Treatment parameters	Storage conditions	Quality changes	Sources
Blueberry juice	200 MPa, 15 min	Tested right after treatment	Total phenolic and anthocyanin content increased, whereas no changes in antioxidant capacity, pH, °Brix and Colors	Barba et al. 2011
Blueberry juice	400-600 MPa, 15 min	Tested right after treatment	Total phenolic and anthocyanin content increased; no changes in pH, °Brix and Colors; but antioxidant capacity decreased	Barba et al. 2011
Blood orange juice	400-600 MPa, 15 min	4ºC for 10 days	93.4% retention rate of anthocyanin; 85% retention rate of ascorbic acid	Torres et al. 2011

Table 2. Examples of HHP effect on quality attributes of fruit products

3.1.2 Pulsed electric field (PEF)

Pulsed electric field processing (PEF) applies short bursts of high voltage electricity for microbial inactivation and causes no or minimum effect on food quality attributes. Briefly, the foods being treated by PEF are placed between two electrodes, usually at room temperature. The applied high voltage results in an electric field that causes microbial inactivation. The applied high voltage is usually in the order of 20-80 kV for microseconds. The common types of electrical field waveform applied include exponentially decaying and square wave (Knorr et al. 1994, Zhang et al. 1995, Barbosa-Cánovas et al. 1999).

The **principles** of PEF processing have been explained by several theories including the trans-membrane potential theory, electromechanical compression theory and the osmotic imbalance theory. One of the most accepted theories is associated with the electroporation of cell membranes. It is generally believed that electric fields induce structural changes in the membranes of microbial cells based on generation of pores of the cell membrane, leading consequently to microbial destruction and inactivation (Tsong 1991, Barbosa-Cánovas et al. 1999).

Compared with thermal processing, PEF processing has many **advantages**. It can preserve the original sensory and nutritional characteristics of foods due to the very short processing time and low processing temperatures. Energy savings for PEF processing are also important compared with conventional thermal processing. Moreover, it is environmentally friendly with no waste generated (Toepfl et al. 2006). Due to these advantages, PEF processing has been widely used in food product preservation including fruit and beverages in the areas of microbial inactivation (Table 3) and shelf-life extension (Table 4).

Foods	Microorganism	Treatment parameters	*Log* reduction	Sources
Apple juice	*E. coli* 8739, *Escherichia coli* O157: H7	30kV/cm, 172μs, <35ºC	5	Evrendilek et al., 1999
Cranberry juice	Total aerobic count, molds, yeasts	40kV/cm, 150μs, <25ºC	4	Jin & Zhang 1999
Orange juice	*Listeria innocua*	30kV/cm, 12μs, 54ºC	6.0	McDonald et al. 2000
Apple cider	*Escherichia coli* O157: H7	90kV/cm, 20μs, 42ºC	5.9	Iu et al. 2001
Orange juice	*Salmonella typhimurium*	90kV/cm, 100μs, 55ºC	5.9	Liang et al., 2002
Apple juice	*Escherichia coli*	34kV/cm, 7.68μs, 55ºC	6.2	Heinz et al. 2003
Grape juice	*Escherichia coli*	34kV/cm, 7.68μs, 55ºC	6.4	Heinz et al. 2003
Cherry juice,	*Penicillum expansum*	34kV/cm, 163μs, 21ºC	100% inaction of spore germination	Evrendilek et al. 2008
Peach nectar	*Penicillum expansum*	34kV/cm, 163μs, 21ºC	100% inaction of spore germination	Evrendilek et al. 2008
Apricot nectar	*Penicillum expansum*	34kV/cm, 163μs, 21ºC	100% inaction of spore germination	Evrendilek et al. 2008

Table 3. Examples of PEF inactivation of microorganisms in fruit products

Foods	Treatment parameters	Storage conditions	Quality changes	Sources
Cranberry juice	40kV/cm, 150µs, <25ºC	4C for 14 days	No changes in color and volatile profile	Jin & Zhang 1999
Apple Juice	35kV/cm, 94µs,	4, 22 and 37ºC for 36 days	No changes in color and Vitamin C	Evrendilek et al. 2000
Apple cider	35kV/cm, 94µs,	4, 22 and 37ºC for 14 days	No changes in color and Vitamin C	Evrendilek et al. 2000
Orange juice	40kV/cm, 97ms, 45ºC	4ºC for 196 days	PEF-processed juice retained more ascorbic acid, flavor, and color than thermally processed juice	Min et al. 2003
Citrus juice	28kV/cm, 100µs, <34ºC	Tested right after treatment	No changes in pH, Brix, electric conductivity, viscosity, non-enzymatic browning index (NEBI), hydroxymethylfurfural (HMF), color, organic acid content, and volatile flavour compounds	Cserhalmi, et al. 2006
Apple juice	36kV/cm, 190µs, <34ºC	Tested right after treatment	PEF preserved better the pH than HTST.	Charles-Rodriguez et al. 2007
Apple juice	35kV/cm, 6.4ms	Tested right after treatment	PEF preserved better the pH, total acidity, phenolics content, and volatile compounds than HTST	Aguilar-Rosas et al. 2007
Water melon juice	35kV/cm, 50µs, <40ºC	Tested right after treatment	113% of Lycopene content, 72% of vitamin C and 100% of antioxidant capacity retention were obtained	Oms-Oliu et al. 2009

Table 4. Examples of PEF effect on quality attributes of fruit products

3.1.3 Ultrasound (US)

Power ultrasound (US) has emerged as a potential non-thermal technique for preservation of food products over the last decade. Compared with diagnostic ultrasound, power US uses a lower frequency range of 20 to 100 kHz and a higher sound intensity of 10 to 1000 W/cm^2 (Baumann et al. 2005).

The **principle** of ultrasonic processing could be explained as follows: Firstly, the ultrasonic transducers convert electrical energy to sound energy. Secondly, when the ultrasonic waves propagate in liquid, small bubbles will be formed and collapsed thousands of times per second. This rapid collapse of the bubbles (cavitation) results in high localized temperatures and pressure, causing breakdown of cell walls, disruption of cell membranes and damage of DNA (Manvell, 1997, Knorr et al. 2004, O'Donnell et al. 2010).

The **application** of high power ultrasound in the food industry has been widely investigated. To meet the FDA requirement of a 5-*log* reduction of microorganisms, a combination of sonication with mild heat treatment and /or pressure is essential (Baumann et al. 2005, D'Amico et al. 2006, Ugarte-Romero et al. 2006, Salleh-Mack and Roberts 2007, Tiwari et al. 2009). Several works have been done to examine the

Foods	Microorganism	Treatment parameters	*Log* reduction	Sources
Carrot juice	*E. coli* K12	19.3 kHz, 700-800 W, 1 min, 60ºC	2.5	Zenker et al. 2003
Apple cider	*Listeria monocytogenes*	48 kHz, 600 W, 3 min, 25ºC	1-2	Rodgers & Ryser 2004
Apple cider	*Escherichia coli* O157: H7	48 kHz, 600 W, 5 min, 25ºC	1-2	Rodgers & Ryser 2004
Apple cider	*Listeria monocytogenes*	20 kHz, 750 W, 5 min, 0.46 W/mL, 60ºC	5	Baumann et al. 2005
Apple cider	*Escherichia coli* O157: H7	20 kHz, 150 W, 18 min, 118 W/cm2, 57ºC	6	D' Amico et al. 2006
Orange juice	Total mesophilic aerobes	500 kHz, 240 W, 15 min, 60ºC	3.4	Valero et al. 2007
Apple juice	*Alicyclobacillus acidoterrestris*	24 kHz, 300 W, 60 min	80%	Yuan et al. 2009
Orange juice	Aerobic mesophilic count (AMC)	20kHz, 500W, 8 min, 89.25μm, 10ºC	1.38	Gomez-Lopez 2010
Orange juice	Yeast and mold counts (YMC)	20 kHz, 500 W, 8 min, 89.25 μm, 10ºC	0.56	Gomez-Lopez 2010

Table 5. Examples of US inactivation of microorganisms in fruit products

Foods	Treatment parameters	Storage conditions	Quality changes	Sources
Carrot juice	19.3 kHz, 700-800 W, 1 min, 60ºC	4ºC for 35 days	Improvement in surface color stability and L-ascorbic acid retention	Zenker et al. 2003
Apple cider	20 kHz, 750 W, 4 min, 0.46 W/mL, 60ºC	Tested right after treatment	Titratable acidity, pH, and °Brix of the cider were not affected.	Ugarte-Romero et al. 2006
Orange juice	500 kHz, 240 W, 15 min, 60ºC	5ºC for 14 days	No detrimental effects on limonin content, brown pigments and colour	Valero et al. 2007
Orange juice	20 kHz, 1500 W, 10 min, 32-38ºC	10C for 30 days	No significant changes in °Brix and titratable acidity. Significant changes in juice pH, colour, non-enzymatic browning, cloud value and ascorbic acid	Tiwari et al. 2009
Orange juice	20 kHz, 500 W, 8 min, 89.25μm, 10ºC	4ºC for 10 days	Color and ascorbic acid content were affected during storage	Gomez-Lopez 2010

Table 6. Examples of US effect on quality attributes of fruit products

effectiveness of ultrasound on inactivation of microorganisms in fruit juices (Table 5). A few studies have been conducted to examine the effect of ultrasound on quality of US-treated fruit juices (Table 6).

Except HHP, PEF and power US, other non-thermal techniques including high pressure homogenization (HPH), membrane filtration and UV-light, among others, are also being investigated.

3.1.4 Ultraviolet light

Ultraviolet light (UV-light) technology utilizes radiation with the electro-magnetic spectrum in the range of 100 to 400 nanometers, between visible light and x-rays. It could be further divided into UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm). UV-C is known to have biocidal effects and destroys microorganisms by degrading their cell walls and DNA (Ngadi et al. 2003). Therefore, UV-C could be used for the inactivation of microorganisms such as bacteria, yeasts, moulds, among others (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). The amount of cell damage depends on the type of medium, microorganisms and the applied UV dose (Ngadi et al. 2003). For fruit juice and beverage processing, the wavelength of 254 nm is widely used (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

As a non-thermal preservation method, UV-C treatment takes the **advantages** of no toxic or significant non-toxic by-products being formed during the treatment, very little energy being required when compared to thermal pasteurization processes, and maximum aroma and color of the treated fruits being maintained (Tran & Farid, 2004).

UV-C treatment has been successfully **applied** to reduce the microbial load in different fruit juices and nectars. Under suitable treatment conditions, more than 5-*log* reduction of some pathogenic microorganism, such as *E. coli*, in fruit juices could be achieved (Guerrero-Beltrán and Barbosa-Cánovas 2004, Keyser et al. 2008). The minimum treatment condition for clear apple juice was under UV dosage of 230 J L^{-1}, whereas higher UV dosage levels would be needed for cloudy juices such as orange juice and tropical juices (Keyser et al. 2008).

3.1.5 High pressure homogenization (HPH)

High pressure homogenization (HPH) is considered to be one of the most promising non-thermal technologies proposed for preservation of fruit juice and beverages. The primary mechanisms of HPH has been identified as a combination of spatial pressure and velocity gradients, turbulence, impingement, cavitation and viscous shear, which leads to the microbial cell disruption and food constituent modification during the HPH process. HPH has shown its ability to increase the safety and shelf-life of fruit juices including orange juice (Lacroix et al. 2005; Tahiri et al. 2006; Welti-Chanes et al. 2009), apple juice (Kumar et al. 2009; Pathanibul et al. 2009) and apricot juice (Patrignani et al. 2009). The effectiveness of the treatment depends on many parameters including processing factors such as pressure, temperature, number of passes and medium factors such as type of juice and microorganisms. For example, up to 350 MPa processing pressure was required to achieve an equivalent 5-*log* inactivation of *L. Innocua*; however, less pressure is required for *E. coli* (> 250 Mpa) in carrot juices (Pathanibul et al. 2009). Another instance is that a higher reduction of *Saccharomyces cerevisiae* 635 was observed in carrot juice (5-*log* reduction) than in apricot juice. (3-*log* reduction) with a pressure level of 100 MPa for up to 8 passes (Patrignani et al. 2009).

3.1.6 Membrane filtration

Ultrafiltration (UF) and microfiltration (MF) are the most commonly used membrane filtration techniques for fruit juice processing. They have been used commercially for the clarification of fruit juices. Through this processing, a "pasteurized" product could be produced with flavours better than thermally treated products (Tallarico et al. 1998; Ortega-Rivas et al. 1998; Cassano et al. 2003). The effectiveness of the treatment depends on many parameters including processing factors such as types of membrane, pore size, transmembrane pressure and medium factors such as type of juice and microorganism. For example, an ultrafiltration (UF) unit, with polysulphone membranes of 10 kDa and 50 kDa pore sizes and trans-membrane pressures of up to 155 kPa, were used to treat apple juices. Results indicated that pH, acid content, and soluble solids did not change but presented less variability for the smaller pore membrane treatment. Relative colour changes were observed for both membranes, which was more detectable for the larger pore membrane treatment (Zarate-Rodriguez et al. 2001). Another application example was to use an ultrafiltration membrane of 15 kDa pore size to filter carrot and citrus juices. Then the clarified juices could be further processed by reverse osmosis and osmotic distillation (Cassano et al. 2003).

3.2 Chemical methods (natural antimicrobials)

Apart from physical methods, some chemical preservatives are widely used for the shelf-life extension of fruit juices and beverages. The most commonly used preservatives are potassium sorbate and sodium benzoate. However, consumer demand for natural origin, safe and environmental friendly food preservatives is increasing. Natural antimicrobials such as *bacteriocins, lactoperoxidase*, herb leaves and oils, spices, chitozan and organic acids have shown feasibility for use in some food products (Gould 2001, Corbo et al. 2009). Some of them have been considered as Generally Recognized As Safe (GRAS) additives in foods. Table 7 lists some natural antimicrobials and their status for GRAS.

3.2.1 Bacteriocins

Bacteriocins are series of antimicrobial peptides which are readily degraded by proteolytic enzymes in the human body. Among them, nisin is the most commonly used food preservative and the GRAS additives permitted by the Food Additive Status List (USFDA, 2006). Apart from dairy, it has been used to preserve fruit and vegetable juices (Yuste & Fung 2004, Settanni & Corsetti, 2008).

3.2.2 Lactoperoxidase

Lactoperoxidase is an enzyme that is widely distributed in colostrum, raw milk and other body liquid. It is an oxidoreductase and catalyses the oxidation of thiocyanate with the consumption of H_2O_2, to produce intermediate products with antibacterial properties (Corbo et al. 2009). These products have been indicated to be bactericidal for some spoilage and pathogenic microorganisms and yeasts (Gould 2001). Not much information had been found on the application of lactoperoxidase in fruit juices. Until recently, it was used for the preservation of tomato juice and mongo fruits (Touch et al. 2004, Le Nguyen et al. 2005).

3.2.3 Herb, spice and flavor oils

Some herbs and spices contain essential oils, which are natural antimicrobials. The main elements of these antimicrobials are phenolic compounds, including caffeic, cinnamic, ferulic and gallic acids, oleuropein, thymol and eugenol (Gould 2001).

Name	Origin	GRAS status
Bay leaves	Plant	Yes
Chitozan	Animal	No
Cinamon	Plant	Yes
Clove	Plant	Yes
Coriander	Plant	No
Garlic	Plant	Yes
Lactoperoxidase	Animal	No
Lemon (peel, balm, grass)	Plant	Yes
Lime	Plant	Yes
Nisin	Microorganism	Yes
Onion	Plant	Yes
Rosemary	Plant	Yes
Sage	Plant	Yes
Thyme	Plant	Yes

* Revised from USFDA (2006): Food Additive Status List

Table 7. Selected natural antimicrobials and their status for GRAS additives*

Among them, sage (*Salvia officinalis*), rosemary (*Rosemarinus officinalis*), clove (*Eugenia aromatica*), coriander (*Coriandrum sativum*), garlic (*Allium sativum*) and onion (*Allium cepa*)) were listed as potential antimicrobials for food use (Deans and Ritchie 1987). The oils of bay leaves, cinnamon, clove and thyme were also proven to be highly effective for food pathogenic microorganisms including *Campylobacter jejuni, Salmonella enteritidis, Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* (Smith-Palmer et al. 1998). It is believed that Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria.

Cinnamon as an antimicrobial agent has been used in apple juice (Yuste and Fung 2004; Friedman et al. 2004), apple cider (Ceylan et al. 2004) and fresh-cut apple slices (Muthuswamy et al. 2008). Ground cinnamon (0.3%) could inhibit the growth of *Staphylococcus aureus*, *Y. enterocolitica* and *Salmonella typhimurium* in apple juice (Yuste and Fung 2004), whereas oils of cinnamon leaf or bark inactivated *Salmonella enterica* and *E. coli* O157:H7 in apple juice (Friedman et al.2004). Ethanol extract of cinnamon bark (1% to 2% w/v) and cinnamic aldehyde (2 mM) could reduce *E. coli* O157:H7 and *L. innocua in vitro*. Ethanol extract of cinnamon bark (1% w/v) reduced significantly the aerobic growth of bacteria inoculated in fresh-cut apples during storage at 6ºC up to 12 days. It was also found that cinnamic aldehyde has greater antimicrobial activity than potassium sorbate (Muthuswamy et al. 2008).

Citrus fruits extracts have also been applied successfully to fruits and vegetables (Fisher & Phillips, 2008). For example, lemon extract was applied for the inhibition of some spoilage microorganisms, such as *Bacillus licheniformis, Lactobacillus spp., Pichia subpelliculosa, Saccharomyces cerevisiae and Candida lusitaniae*, the minimum inhibition concentration is 100 to 150 ppm (Conte et al. 2007). The growth of pathogenic bacteria, *Escherichia coli* O157:H7, *Listeria innocua* and the food spoilage fungus, *Penicillium chrysogenum* were suppressed by three phenolic compounds (catechin, chlorogenic acid and phloridzin) at 25 mM but the growth of food spoilage yeast *Saccharomyces cerevisiae* was inhibited only by chlorogenic acid and phloridzin (Muthuswamy and Rupasinghe, 2007). Vanillin, the predominant phenolic compound present in vanilla beans, has shown a concentration dependent response and the minimal inhibitory concentration (MIC) of 6 to 18 mM for pathogenic and spoilage microorganisms (Rupasinghe et al., 2006).

3.2.4 Chitozan

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1 → 4)-N-acetyl-D-glucosamine] (No & Meyers, 1995). It is widely produced from crab, shrimp and crawfish, with different deacetylation grades and molecular weights which contribute to different functionalities (No et al., 2007).

Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of microorganisms (Sagoo and others 2002). The principles of the antimicrobial activity of chitosan could be explained by several hypotheses. One hypothesis is that the positively charged chitosan molecules could interact with the negatively charged microbial cell membranes, which would affect the cell permeability and lead to the leakage of intracellular compounds (Fang et al., 1994). Another hypothesis is that the interaction of diffused hydrolysis substances with microbial DNA could lead to the inhibition of the mRNA and protein synthesis of the microorganisms (Sudarshan et al., 1992).

A limited works have been done to assess the antimicrobial properties of chitosan in fruit juices (Roller and Covill 1999; Rhoades and Roller 2000). Chitosan glutamate was reported to be an effective preservative against spoilage yeasts in apple juice. Chitosan glutamate in apple juice from 0.1 to 5 g/L inhibited the growth of all spoilage yeasts at 25°C. The most sensitive strain, *Z. bailii*, was completely inactivated by chitosan at 0.1 and 0.4 g/L for 32-day of storage at 25°C. The most resistant strain, *S. ludwigii*, required 5 g/L of chitosan for complete inactivation and for maintaining yeast-free conditions in apple juice for 14 days at 25°C (Roller and Covill 1999). Another study by Rhoades and Roller (2000) showed that 0.3 g/L of Chitosan eliminated all the yeasts in pasteurized apple-elderflower juice during a 13-day of storage at 7°C. However, the total bacterial counts and the lactic acid bacterial counts increased slower than the control (Rhoades and Roller 2000).

Foods	Microorganism	Method used	*Log* reduction	Sources
Apple cider	*Escherichia coli* O157:H7	PEF (90 kV/cm, 20 µs, 42ºC) with nisin or cinamon	6-8	Iu et al. 2001
Orange juice	Natural microflora	PEF (80 kV/cm, 44ºC) with Nisin (100 U/ml)	6	Hodgins et al. 2002
Orange juice	*Escherichia coli* O157:H7	PEF (90 kV/cm, 100 µs, 55ºC) with nisin and/or lysozyme	> 7	Liang et al. 2002
Apple cider	*Listeria monocytogenes*	US (48 kHz, 600 W, 5 min, 25ºC) with copper, sodium hypochlorite	5	Rodgers and Ryser 2004
Apple cider	*Escherichia coli* O157: H7	US (20 kHz, 150 W, 18 min, 118 W/cm2) with heat (57ºC)	> 7	D' Amico et al. 2006
Carrot juice	*Listeria innocua*	HPH (350 MPa) with nisin (10 IU/ml)	> 5	Pathanibul et al. 2009
Strawberry juice	*Escherichia coli* O157: H7 and *Salmonella Enteritidis*	PEF (35 KV/cm, 4 µs, <40ºC) with 0.05% cinnamon bark oil or 0.5% citric acid	>5	Mosqueda-Melgar et al. 2008
Apple juice	*Escherichia coli* O157: H7 and *Salmonella Enteritidis*	PEF (35 KV/cm, 4 µs, <40ºC) with 0.1% cinnamon bark oil or 1.5% citric acid	>5	Mosqueda-Melgar et al. 2008
Pear Juice	*Escherichia coli* O157: H7 and *Salmonella Enteritidis*	PEF (35 KV/cm, 4 µs, <40ºC) with 0.1% cinnamon bark oil or 1.5% citric acid	>5	Mosqueda-Melgar et al. 2008
Strawberry juice	*Escherichia coli* O157: H7 and *Salmonella Enteritidis*	PEF (18.6 KV/cm, 150 µs, 55ºC) with 750 ppm sodium benzoate and 350 ppm potassium sorbate	5.11	Gurtler et al. 2011
Strawberry juice	*Escherichia coli* O157: H7 and *Salmonella Enteritidis*	PEF (18.6 KV/cm, 150 µs, 55ºC) with 750 ppm sodium benzoate, 350 ppm potassium sorbate and 2.7% citric acid	6.95	Gurtler et al. 2011

Table 8. Preservation of fruit juices by combination methods

Chitosan has been approved as a food additive in Japan in 1983 and in Korea in 1995. However, it is so far not a GRAS approved food additive by the FDA. As long as receiving the FDA approval for GRAS status, Chitosan as a food additive and its applications in food systems will certainly have a brighter future.

3.3 Combination of physical and chemical methods

It is proved that some individual non-thermal methods as well as natural antimicrobials are effective in inactivating microorganisms and at the same time do not adversely affect the sensory and nutritional quality of the fruit juice and other products. Moreover, the combination of these techniques could provide synergistic effects on prolonging the fruit juice shelf-life and potentially as replacement for traditional pasteurization methods. Table 8 and Table 9 list some examples of recent progresses in these combined techniques for the microbial inactivation (Table 8) and shelf-life extension (Table 9) of fruit juices and beverages.

Foods	Method used	Storage conditions	Quality changes	Sources
Apple cider	US (20 kHz, 750W, 4 min, 0.46W/mL) with heat (60ºC)	Tested right after treatment	Titratable acidity, pH, and °Brix of the cider were not affected.	Ugarte-Romero et al. 2006
Apple juice	UV (5.3 J/cm²) with PEF (34 kV/cm, 98 μs) or with US (20 kHz, 750 W, 5 bar, 43ºC)	Tested right after treatment	No significant impact on non-enzymatic browning, total phenolics, antioxidant activity, flavor, color and odor of the juices.	Caminiti et al. 2011
Cranberry juice	UV (5.3 J/cm²) with PEF (34 kV/cm, 98 μs) or with US (20 kHz, 750 W, 5 bar, 43ºC)	Tested right after treatment	No significant impact on non-enzymatic browning, total phenolics, antioxidant activity, flavor, color and odor of the juices.	Caminiti et al. 2011

Table 9. Examples of combined preservation methods on quality attributes of fruit products

4. Conclusions

Non-thermal processing is a promising and useful approach for fruit juice and beverage preservation. The products based on these techniques show many advantages such as the retention of sensorial qualities and nutritional values over traditional thermal processing. However, among these non-thermal techniques, only high pressure processing has been adopted by the food industry so far. Additional pilot-scale testing may require for these non-thermal preservation methods to become a real alternative for thermal processing.

Similarly, the application of natural antimicrobial compounds in fruit juice and beverages is in the laboratory scale. But the potential benefits of these compounds would lead to a fast growth of scale-up and commercial application in food industry.

More practically, the combination of non-thermal techniques and natural antimicrobial compounds would be the future trends for fruit juice and beverages preservation, due to the proven records for effective inhibition of microorganisms and shelf-life extension of fruit juices and beverages.

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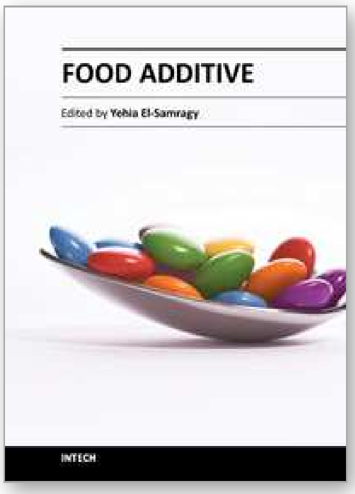

Food Additive
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A food additive is defined as a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value. Food additives are natural or manufactured substances, which are added to food to restore colors lost during processing. They provide sweetness, prevent deterioration during storage and guard against food poisoning (preservatives). This book provides a review of traditional and non-traditional food preservation approaches and ingredients used as food additives. It also provides detailed knowledge for the evaluation of the agro-industrial wastes based on their great potential for the production of industrially relevant food additives. Furthermore the assessment of potential reproductive and developmental toxicity perspectives of some newly synthesized food additives on market has been covered. Finally, the identification of the areas relevant for future research has been pointed out indicating that there is more and more information needed to explore the possibility of the implementation of some other materials to be used as food additives.

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