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Review

High hydrostatic pressure (HHP) inactivation of foodborne pathogens in low-acid juices

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Summary Consumption of unpasteurised fruit/vegetable juices has increased in recent years due to their freshness, low calorie contribution and good nutritional quality. However, unpasteurised fresh juices with low acidity ($pH > 4.6$) and high water activity ($a_w > 0.85$) can support the growth of pathogens. Hence, pasteurisation is a necessary process in the production of low-acid juices. Consumer demand has required minimally processed high-quality foods that are free from additives, that are fresh tasting and microbiologically safe, and with an extended shelf life. High hydrostatic pressure (HHP) treatment is considered to be an alternative to thermal pasteurisation for fruit and vegetable juices. HHP treatment could preserve nutritional value and the sensory properties of fruits and vegetables due to its limited effect on the covalent bonds of low-molecular-mass compounds such as colour, flavour compounds and vitamins. However, inactivation of important foodborne pathogens in low-acid foods by HHP is most urgent and critical. More research should be performed in order to satisfy consumer demands for fresh-tasting products while retaining safety.

Keywords Cantaloupe, carrot, foodborne outbreak, foodborne pathogens, high hydrostatic pressure, low-acid juice, tomato, watermelon.

Introduction

Consumers are currently modifying their eating habits and becoming aware of the relationship between diet and disease prevention. The intake of minimally processed food products such as fruit and vegetable juices is recommended for a healthy diet and various health effects (Song *et al.*, 2006; Sousa *et al.*, 2012). Consumption of unpasteurised fruit and vegetable juices has increased in the recent years due to their characteristics such as freshness, high vitamins content, low calorie contribution and good nutritional quality (Raybaudi-Massilia *et al.*, 2009). However, unpasteurised fresh juices with low acidity ($pH > 4.6$) and high water activity ($a_w > 0.85$) can support the growth of a variety of pathogenic microorganisms. Enteric foodborne pathogens including *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium, and Gram-positive pathogens such as *Listeria monocytogenes* have all been reported to be capable of survival in raw fruit and vegetable juices (Pathanibul *et al.*, 2009). Hence, pasteurisation is a necessary process in the production of low-acid juices. However, traditional thermal treatments adversely affect the organoleptic and nutritional properties of foods (Wolbang *et al.*, 2008). Colour, flavour and texture are important quality characteristics of fruits and vegetables and major factors affecting the sensory perception and consumer acceptance of foods (Oey *et al.*, 2008).

Watermelon is a low-acid food and a thermo-sensitive fruit whose organoleptic and nutritional properties will be destroyed by heat. Lycopene loss of watermelon juice was also reported during thermal treatment and storage (Zhang *et al.*, 2011; Liu *et al.*, 2012). Hami melon or cantaloupe is a favoured fruit for its nutritional quality and unique aroma. However, freshly squeezed cantaloupe juice has a very short shelf life and the juice is heat sensitive. The sensitive nutrients, colour and aromatic profile will be spoiled greatly or cooked off-odour will be formed when it was produced with thermal treatment (Chen *et al.*, 2010; Ma *et al.*, 2010). Among common fruits and vegetables, carrots are the most preferred for their high content of fibres, carotenoids, vitamins C and E, and phenolics (Potter *et al.*, 2011). Carrots have high b-carotene content, and carrot juice has high potential as a natural source of β-carotene. Untreated, raw carrot juice has a short shelf life and should normally be consumed within 1–2 days. Heat processing can be used to extend the shelf life and improve the safety of the juice, but it has been well documented that heating is

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detrimental to its organoleptic and nutritional quality. Heat treatment could destroy carotenoids, cause colour changes, and reduce the ascorbic acid content and the free-radical scavenging activity of carrot juice (Kim *et al.*, 2001; Dede *et al.*, 2007; Patterson *et al.*, 2012). Effects of thermal processing on the processing qualities of tomato juices had also been investigated. The volatile components and vitamin C of canned tomato juice was reduced by thermal treatments. The colour of tomato juice degraded more rapidly with increasing temperature. Lycopene in tomato is relatively resistant to thermal degradation, whereas other antioxidants (ascorbic acid, tocopherol and β-carotene) degrade more rapidly by thermal processing (Hsu, 2008).

Consumer demand has required minimally processed high-quality foods that are free from additives, that are fresh tasting and microbiologically safe, and with an extended shelf life (Patterson, 2005). To achieve this balance between food quality and safety, there is a need to optimise conventional processing techniques currently applied in food industries and to develop novel processing techniques (Oey *et al.*, 2008). Due to this demand, interest in non-thermal pasteurisation of juices has been increasing and the most investigated non-thermal pasteurisation techniques include high hydrostatic pressure (HHP), pulsed electric fields, oscillating magnetic fields, intense light pulses, irradiation, ultrasonication or combinations of these methods (Liao *et al.*, 2010). Among these, HHP treatment is considered to be a promising alternative to thermal pasteurisation for fruit and vegetable juices used alone, or when used in combination with traditional techniques (Jordan *et al.*, 2001). This technology in the foods area ensures a high quality level of the products (flavour, colour, vitamin content, biologically active components, etc.) similar to that of the fresh raw material (Houška *et al.*, 2006). HHP treatment could preserve nutritional value and the delicate sensory properties of fruits and vegetables due to its limited effect on the covalent bonds of low-molecular-mass compounds such as colour, flavour compounds and vitamins (Oey *et al.*, 2008).

Currently HHP-processed products are low pH fruit juices (grapefruit juice, mandarin juice, apple and orange juice), jams, jellies, fruit dressing, avocado, yoghurt, salted raw squid, fish sausages and oysters (Ohlsson, 2002). With respect to microbiological safety, quantifying inactivation of important food-related pathogens by high pressure is most urgent and critical in the establishment of high-pressure processing, especially in low-acid foods (Van Opstal *et al.*, 2005).

This paper aims at giving a thorough overview of the most recent findings specifically on how HHP treatments are used for the inactivation of foodborne pathogens in low-acid fruit and vegetable juices. In addition, special attention is given to the possible impacts of HHP on quality parameters of low-acid juices.

Health significance of low-acid juices

Food processors who wish to use HHP to preserve foods would benefit from a specified limited number of pressure–time combinations. These combinations have been proved to inactivate the ‘pertinent microorganism’ (most resistant microorganism of public health significance that is likely to occur in the juice and is the pathogen that one must target for) by $5\log_{10}$. By choosing the most resistant pathogen as target, they can also treat the product for all other pathogens that are less resistant to the extent of the treatment (U.S. FDA, 2004). According to Juice HACCP Hazards and Controls Guidance, low-acid juices, such as carrot juice, are not subjected to the low-acid canned foods regulation and they need to be distributed under refrigeration. Therefore, selection of pressure-resistant strains of foodborne pathogens, such as *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp. or *Staphylococcus* spp., will also apply for low-acid juices.

Carrot juice is one of the most popular vegetable juices (pH > 6.0). As carrot juice is a low-acid food, it has a higher risk of bacterial contamination than the other acidic foods (Zhou *et al.*, 2009). Tomatoes and tomato products having a pH value of 4.7 are also not subjected to the low-acid canned foods regulation (U.S. CFR, 2012). Melon and watermelon products (pH 5.2–6.7, a_w 0.97–0.99) are regarded as potentially hazardous foods by the Food and Drug Administration. Outbreaks of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* have been linked with the consumption of fresh-cut as well as juices of melon and watermelon. *E. coli* has been identified as the pathogen in several outbreaks involving carrots since 1990 (OMAF, 2001; Mosqueda-Melgar *et al.*, 2007). Selected outbreaks involving melon, watermelon and carrots are given in Table 1.

High hydrostatic pressure treatments of low-acid juices

Effect of HHP on microorganisms in cantaloupe juice was studied and demonstrated that they achieved 5-log cycles reductions of *E. coli* within 8 min at 500 MPa at room temperature in melon juice. Sensory evaluation indicated that there was no significant aromatic difference between HHP-treated and untreated cantaloupe juice samples (Ma *et al.*, 2010). Wolbarg *et al.* (2008) studied the effect of high-pressure processing on nutritional value and quality attributes of *Cucumis melo* L. High-pressure processing of 600 MPa for 10 min resulted in higher levels of β-carotene but lower levels of vitamin C. The colour parameters were

Table 1 Selected foodborne outbreaks traced to fresh produce and juices (compiled from OMAF, 2002; CDC, 2012)

Year	Pathogen	Commodity vehicle identified	Number of cases	Area where outbreak occurred
1990	*Salmonella* Chester	Cantaloupe	245	30 states of US
1991	*Salmonella* Javiana	Watermelon	26	Michigan, US
1991	*Salmonella* Poona	Cantaloupe	>400	23 states of US
1993	*Escherichia coli* 0157:H7	Cantaloupe	29	Oregon, US
1993	*Clostridium botulinum*	Carrot juice	1	Washington, US
1993	*Escherichia coli* (ETEC)	Carrots	47	Rhode Island, US
1993	*Escherichia coli* (ETEC)	Carrots	121	New Hampshire, US
1997	*Salmonella* Saphra	Cantaloupe	24	1 state of US
1998	*Salmonella* Oranienburg	Cantaloupe	22	Ontario, US
2000	*Escherichia coli*	Watermelon	>41	Milwaukee, of US
2000	*Salmonella* Poona	Cantaloupe	43	7 states of US
2001	*Salmonella* Poona	Cantaloupe	30	8 states of US
2006	*Salmonella* Typhimurium	Tomato	183	21 states of US
2008	*Salmonella* Litchfield	Cantaloupe	51	16 states of US
2010	*Salmonella* typhi	Mamey fruit pulp	9	2 states of US
2011	*Salmonella* Panama	Cantaloupe	20	10 states of US
2011	*Listeria monocytogenes*	Cantaloupe	146 with 30 deaths	28 states of US

adversely affected by HPP. The effect of thermal, ultraviolet-c and high-pressure treatments on quality parameters of watermelon juice was studied by Zhang *et al*. (2011). High-pressure treatment showed the lowest changes in colour, dynamic viscosity, browning degree and lycopene content of the treated watermelon juice amongst the three treatments.

Jordan *et al.* (2001) investigated methods for inactivating the pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices (orange, apple and tomato juice). Treatment at 500 MPa and 300 MPa for 5 min at room temperature achieved a reduction of 5 log units for *E. coli* O157 and *L. monocytogenes*, respectively. They also achieved a greater level of inactivation in tomato juice than in orange juice of lower pH. It was due to the presence of low levels (0.7%) of salt in the tomato juice. Gupta *et al.* (2010) determined the storage stability of lycopene in tomato juice subjected to combined pressure–heat treatments. Samples were subjected to pressure-assisted thermal processing (PATP; 600 MPa, 100 °C, 10 min), high-pressure processing (HPP; 700 MPa, 45 °C, 10 min) and thermal processing (TP; 0.1 MPa, 100 °C, 35 min). Processed samples were stored at 4, 25 and 37 °C for up to 52 weeks. HPP and PATP treatments significantly improved the extractability of lycopene over TP and control. HPP- and PATP-processed samples better retained total lycopene during storage. HPP- and PATP-processed samples also showed better colour retention as compared to TP samples.

Effects of combined treatment of high hydrostatic pressure and mild heat on the quality of carrot juice were studied by Kim *et al.* (2001). About 95% of food-quality-related enzymes were lost at 400 MPa and 70 °C, for 10 min, while α- and β-carotene were relatively stable at the combined process. These results indicated that the combined treatment of high pressure and mild heat could be used as an effective process for the production of high-quality carrot juice.

Pilavtepe-Çelik *et al.* (2009) developed a new mathematical model for the inactivation of *Escherichia coli* O157:H7 and *Staphylococcus aureus* by HHP in carrot juice and peptone water. Simulations of 5 log10 reductions of both pathogens in both media indicated that carrot juice had a protective effect on *E. coli* O157:H7 whereas it had a sensitising effect on *S. aureus*. Phytoalexins were defined as the low-molecular-weight antimicrobial compounds produced by plants in response to the infection by microorganisms. 6-Methoxymellein was identified as a common phytoalexin produced by carrot roots and had a broad antimicrobial spectrum. This may be due to the presence of antimicrobial compounds, such as phytoalexins, in the carrots. In general, Gram-positive bacteria are reported to be more sensitive to 6-methoxymellein than Gram-negative bacteria. Patterson *et al.* (2012) studied the effect of HPP on the microbiological quality and safety of carrot juice during refrigerated storage. When the inoculated carrot juice was pressure-treated (500 MPa per min per 20 °C), no *L. monocytogenes* were found immediately after pressure treatment or during storage at 4, 8 and 12 °C (>6 log inactivation). In contrast, pressure treatment in TSBYE only resulted in 1.65 log inactivation and survivors grew rapidly. This suggests that there is a synergistic effect between the antilisterial properties of the carrot juice and HPP.

Conclusions

High hydrostatic pressure treatment is capable of securing the freshness, nutritional value and organoleptic properties of low-acid juices. In terms of these properties, we can say that HHP treatment is an alternative to thermal heat treatment due to its limited effect. There is more research on nutritional and sensory quality of HHP-treated low-acid juices; however, there is limited research on the inactivation of foodborne pathogens by HHP (especially in vegetable juices).

In general, under similar pressurisation conditions, microbial destruction is lower in a food system than in a buffer or bacteriological medium. However, food systems are not always more protective. Different food constituents and ingredients can play a protective role against pressure. Carbohydrates are generally more baroprotective than salts. The effect of fat content was not clear: in some cases, either there was no baroprotection or increasing fat content did not result in increasing baroprotection (Ray *et al.*, 2001; Mor-Mur & Yuste, 2005). The chemical composition of the substrate during treatment can also have a significant effect on the response of microorganisms to pressure. Inactivation data obtained using buffers or laboratory media, therefore, should not be extrapolated to real food situations (Patterson, 2005). The studies especially in carrot and tomato juice revealed that food systems can exhibit either protective or sensitising effect on microorganisms. More research should be performed in low-acid juices in order to satisfy consumer demands for fresh-tasting products while retaining safety.

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