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Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices

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Abstract

The purpose of this study was to investigate the effect of high hydrostatic pressure with a mild heat treatment on *Staphylococcus aureus* 485, *Escherichia coli* O157:H7 933 and *Salmonella* Enteritidis FDA in apple, orange, apricot and sour cherry juices. The effectiveness of the treatment on polyphenol oxidase activity in apple juice and pectinesterase activity in orange juice were also determined. An inoculum of microorganisms was completely inactivated at 350 MPa and 40 °C in 5 min. The residual polyphenol oxidase activity in apple juice after treatment at 450 MPa and 50 °C for 60 min was obtained as 9 ± 2.2%. The residual pectinesterase activity in orange juice after treatment at 450 MPa and 50 °C for 30 min was determined as approximately 7 ± 1.6%. It compares with 12 ± 0.2% at a treatment of 40 °C and 450 MPa for 60 min. Pressure resistant isoenzymes were thought to be responsible for the final residual activity. The inactivation is irreversible and the enzyme is not reactivated upon storage. High pressure processing constitutes an effective technology to inactivate the enzymes in fruit juices. Pressures higher than 400 MPa can be combined with mild heat (<50 °C) to accelerate enzyme inactivation.

Keywords: High hydrostatic pressure; *Staphylococcus aureus* 485; *Escherichia coli* O157:H7; *Salmonella* Enteritidis FDA; Polyphenol oxidase; Pectinesterase; Fruit juice

1. Introduction

Fruit juices are susceptible to microbial spoilage and enzymatic activities and thus have a limited shelf life. High hydrostatic pressure (HHP) treatment can be used to inactivate microorganisms and enzymes. The development of reliable and safe HHP processes will require detailed knowledge about the HHP inactivation of pathogens with an adequate margin of safety, and how pressure–time–temperature combinations and the juice type affecting the inactivation (Cheftel, 1992; Smelt, 1998). The studies showed that different strains of a species can have widely varying pressure resistance and the stage of growth of bacteria is important in determining pressure resistance. Cells in stationary phase are more pressure resistant than those in the exponential phase (Benito, Ventoura, Casadei, Robinson, & Mackey, 1999; Isaacs & Chilton, 1995; Patterson, Quinn, Simpson, & Gilmour, 1995). Although food poisoning outbreaks have often been associated with the consumption of foods of animal origin, outbreaks associated with traditionally consumed acid foods were also reported (Cody et al., 1999; McCarthy,

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1996). As pressure resistance of bacteria varies among foods it is important to validate processing parameters in foods and not extrapolate results from buffers and laboratory media. Therefore, in developing pressurization parameters, pressure-resistant strains of bacterial species should be used as serrogates to ensure greater safety of foods. The effect of low temperature, high pressure treatment on the survival of mainly gram-negative foodborne pathogens, *E. coli* O157:H7 and *Salmonella*, inoculated in different fruit juices has been reported in literature (Linton, McClements, & Patterson, 1999; Teo, Ravishankar, & Sizer, 2001). It has also been reported that gram-negative bacteria and cells in exponential growth phase, respectively, are more pressure-sensitive than gram-positive bacteria and early stationary phase cells (Hauben, Wuytac, Soontjens, & Michiels, 1996; Kalchayanand, Sikes, Dunne, & Ray, 1998).

The effect of HHP on the rate and degree of irreversible enzyme denaturation depends on the composition and structure of enzyme, pH, pressure, temperature and the type and concentration of solutes in the liquid that the enzyme presents. Seyderhelm, Boguslawski, Michaelis, and Knorr (1996) ranked the enzymes according to their pressure induced inactivation in the following order (from low to high): lipoxygenase, lactoperoxidase, pectinesterase, lipase, phosphatase catalase, polyphenol oxidase, peroxidase by considering distinct conditions within a pressure range of 0.1–900 MPa, temperatures from 25 to 60 °C, pH 3–7 and time of treatment of 2–45 min in model buffer solutions. Polyphenol oxidase (PPO) is responsible for enzymatic browning in fruit juices. Enzymatic browning starts with the initial enzymatic oxidation of phenols to quinones in the presence of oxygen. Partially purified PPO from apple were noticeably inactivated at pressures above 600 MPa at room temperature and pH 6–7 (Weemaes, Ludikhuyze, Van Den Broeck, & Hendrickx, 1998). According to the study of Gomes and Ledward (1996), apple slices browned to a limited degree on pressure treatment up to 600 MPa and browned still further on subsequent storage. HHP treatment together with a mild heat treatment reduces PPO activities of fruit-derived products such as slices, purees and juices (Cano, Hernandez, & De Ancos, 1997; Castellari, Matricardi, Arfelli, Rovera, & Amati, 1997). Pectinesterase (PE) causes undesired cloud instability in citrus juices. It catalysis the hydrolysis of methyl ester groups from pectin and leads to the formation of a calcium pectate gel that causes the cloud loss. Current studies have been conducted on the application of HHP treatment for the inactivation of PE in order to prevent cloud loss and the results show that HHP treatment is a potentially useful tool for extending the shelf life of citrus juices (Goodner, Braddock, & Parish, 1998; Goodner, Braddock, Parish, & Sims, 1999; Nienaber & Shellhammer, 2001; Ogawa, Fukuhisa, Kuba, & Fukumota, 1990). For efficient inactivation of PE in orange juices, pressures greater than 600 MPa should be used at room temperature.

It is apparent that pressure treatment of fruit juices (pH < 4.5) with a combination of heat treatment is often required for effective microbial and enzyme inactivation. In this study, the efficiency of HHP treatment on inactivation of *S. aureus* 485, *E. coli* O157:H7 933 and *S.* Enteritidis FDA that are relatively resistant to pressure (Alpas et al., 1999) was examined in apple, orange, apricot and sour cherry juices. It was hypothesized that the treatment conditions used to inactivate these organisms would be sufficient to kill other, less resistant pathogens. Also the effectiveness of HHP processing at lower temperatures on PPO activity in apple juice, PE activity in orange juice and on some juice quality factors were determined.

2. Materials and methods

2.1. Fruit juices

Apple (Amasya) and orange (Valencia) juices were freshly squeezed. Apricot and sour cherry juices were obtained from Food Engineering Department of Ankara University (Ankara, Turkey).

2.2. Bacterial strains

S. aureus 485 (from FDA Food Microbiology Laboratory, Washington, D.C.),*E. coli* O157:H7 933 (from M. Doyle, University of Georgia, Griffin) and *S.* Enteritidis FDA (from US Army Food Research Laboratories, Natick, MA) were used in this study. Previous work has shown these strains to be relatively pressure-resistant (Alpas et al., 1999). The strains were cultivated in Tryptic Soy Broth (Difco, Detroit, MI) supplemented with 0.6% yeast extract (Difco; TSBY) at 37 °C for 16–18 h and transferred to fresh broth every 48 h.

2.3. Preparation and inoculation of fruit juices

The fruit juices were autoclaved for 5 min at 121 °C and inoculated with foodborne pathogens (at their early stationary phase) in TSBY to obtain about 10^8 colony forming units (cfu)/ml fruit juice (1 ml of culture into 100 ml fruit juice). The fruit juices with bacteria were dispensed in 2-ml portions in sterile plastic vials (Simport Plastic, Canada) avoiding air bubbles as much as possible. The vials were vortexed for 2 min and vacuum-sealed in sterile plastic bags (Fischer Scientific, USA). After the HHP treatments, Samples were held in ice and all the measurements were done in 1 h.

2.4. HHP system and treatment of fruit juices

HHP treatments were performed in a designed and constructed lab-scale unit (capacity: 30 cm^3, maximum P: 500 MPa). The rate of pressure increase and pressure release was approximately 5–10 s for the designed system. A mixture of deionized water and glycol was used as pressure transmitting medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a means for restraining the end closures, a pressure pump, and a hydraulic unit to generate high pressure for system compression and also a temperature control device. The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome plated and polished to mirror finish (steel type heat treated special K) which was processed into the required sizes at Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. The liquid was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Pressurization time reported in this study did not include the pressure increase and release times.

In pressurization study, the vials containing fresh or sterilized fruit juice samples were exposed to 250–450 MPa pressure for 0–60 min at 25–50 °C depending on enzyme or microbial inactivation. The vials were placed inside the cylindrical vessel of the HHP equipment, the chamber was closed and the samples were kept for 1 and 2 min for temperature equilibration, before pressurization. This temperature and time relation for equilibration had been determined earlier. Immediately after pressurization, the vials were removed and cooled in an ice bath. Unpressurized samples were used as controls. Experiments and measurements were duplicated.

2.5. Determination of microbial counts

After pressure treatment, appropriate serial dilutions were performed in sterile 0.1% peptone solution (Beckon Dickinson and Co., USA). In all cases, duplicate 0.1 ml volumes from the selected dilutions were surface plated onto pre-poured Tryptic Soy Agar (Difco, USA) supplemented with 0.6% yeast extract agar (TSAY) plates. The plates were incubated at 37 °C for 48 h and plates containing 25–250 cfu/ml were selected for counting. Two replicates were performed on separate days and average of eight counts (two cryovials × two plates × two times) was used to calculate the results.

2.6. Measurement of PPO activity in apple juice and PE activity in orange juice

PPO activity in fresh and HHP treated apple juice samples was determined by using catechol as substrate. One unit of enzyme activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001/s under the assay conditions (Özoğlu & Bayındırlı, 2002). PE activity in orange juice was determined by using pectin as substrate (Kimball, 1991). PE unit (PEU) was defined as release of 1 μmole of carboxyl group/min under defined conditions.

2.7. Measurement of browning in apple juice

After treatment, the color was assessed visually (3 persons) and also absorbance values at 420 nm were recorded with an UV spectrophotometer (Shimadzu UV-1202) after centrifugation (Nüve NF 615) at 5000 rpm for 10 min. The change in A_{420} value of unprocessed fresh juice stored at 25 °C for 24 h (completely brown) was accepted as very dark brown and ranked as 5. By visual assessment and also by checking A_{420} change of treated and untreated control samples, the samples were evaluated as 1: no browning, 2: slightly brown, 3:brown, 4: dark brown and 5: very dark brown.

2.8. Measurement of cloud loss in orange juice

Samples of juice were centrifuged at $360 \times g$ for 10 min and then transmission at 650 nm was determined. Light transmission values were interpreted as follows: 0–24%: no cloud loss, 25–35%: slight cloud loss, 36–60%: definite cloud loss, 61–100%: extreme cloud loss.

2.9. Measurement of ascorbic acid in orange juice

It was analyzed by a titrimetric method by using 2,6-dichloroindophenol (AOAC, 1975).

2.10. Statistical analysis

Statistical analysis was carried out using data analysis functions in Microsoft Excel and Significant differences between the results were calculated by analysis of variance (ANOVA). Differences at $p < 0.05$ were considered to be significant.

3. Results and discussion

The effect of HHP treatment on *S. aureus* 485, *E. coli* O157:H7 933 and *S.* Enteritidis FDA in selected fruit juices are presented at Tables 1 and 2. Although they cannot grow at low pH, some certain strains of *E. coli, Shigella* and *Salmonella* species has been reported to survive several days or even weeks in acidic foods (Leyer, Wang, & Johnston, 1995). The level of inactivation of pathogens in food deemed necessary to achieve an adequate margin of safety varies depending on the severity of the hazard posed by the organism, and whether subsequent handling and storage conditions are likely to

Table 1
Effect of HHP treatment at 250 MPa and 30 °C on survival of foodborne pathogens in selected juices[a]

Fruit juice	Time (min)	Log_{10} cfu/ml		
		S. aureus 485 control = 8.20[b]	*E. coli* O157:H7 control = 8.28[b]	*S.* Enteritidis FDA control = 8.53[b]
Apricot juice (pH = 3.80)	5	4.15	3.43	3.75
	10	3.90	3.23	3.32
	20	3.20	2.38	2.50
Orange juice (pH = 3.76)	5	3.95	3.18	3.23
	10	3.68	3.04	3.00
	20	2.83	1.94	1.78
Sour cherry juice (pH = 3.30)	5	3.86	3.00	3.06
	10	3.50	2.53	1.87
	20	2.50	1.43	1.27

[a] Values represent mean ($n = 8$) $\log_{10}$ cfu/ml.
[b] Values represent the $\log_{10}$ cfu/ml of unpressurized control samples.

Table 2
Effect of HHP treatment at 350 MPa and 5 min on survival of foodborne pathogens in selected juices[a]

Fruit juice	T (°C)	Log_{10} cfu/ml		
		S. aureus 485 control = 8.20[b]	*E. coli* O157:H7 control = 8.28[b]	*S.* Enteritidis FDA control = 8.53[b]
Apricot juice (pH = 3.80)	30	1.26	1.00	0.53
	40	ND[c]	ND	ND
Orange juice (pH = 3.76)	30	0.94	0.86	ND
	40	ND	ND	ND
Sour cherry juice (pH = 3.30)	30	0.76	0.61	ND
	40	ND	ND	ND
Apple juice (pH = 3.50)	30	1.40	1.18	0.73
	40	ND	ND	ND

[a] Values represent mean ($n = 8$) $\log_{10}$ cfu/ml.
[b] Values represent the $\log_{10}$ cfu/ml of unpressurized control samples.
[c] ND, not determined: no survivors were detected at the corresponding treatment.

increase the risk. In addition to microbiological inactivation, the economic feasibility of HHP processing requires that treatment conditions be optimized to achieve the lowest pressure/shortest time combinations needed to eliminate pathogens of concern from the food to be treated. Ideally, pressures should be no greater than about 350 MPa to reduce capital equipment costs, although successful commercial applications have used higher pressures than this. A combined treatment of 5 min at 350 MPa, at 40 °C provided a significant viability loss ($p < 0.05$) of more than 8 log cycles for the selected relatively pressure-resistant pathogens in the fruit juices studied.

Jordan, Pascual, Bracey, and Mackey (2001) reported a 5 and 1 log-cycle reduction in viable cell numbers of *E. coli* O157 in apple and orange juice, respectively after treatment at 500 MPa at 20 °C for 5 min. According to the study of Linton et al. (1999), the processing temperature must be considered when treating of fruit juices to ensure microbiological safety. They found that a pressure treatment of 550 MPa for 5 min at 20 °C produced a 6-log inactivation of *E.coli* O157:H7 in orange juice at pH 3.4–4.5 but not such a reduction at pH 5. Increasing the processing temperature to 30 °C provided a 6-log reduction at pH 5. Our results revealed significant reduction ($p < 0.05$) at a much lower pressure (350 MPa) at the higher temperatures of 40 °C. The sensitization of bacterial cells to acid by pressure treatment is probably a general phenomenon but any change in the pH of foods during pressure treatment is very difficult to measure. The pressure treatment injures cells at its own right but also causes changes in the cells that make them more susceptible to subsequent acid injury. The simplest interpretation of the acid sensitization effect would be that a proportion of the cells that survive pressurization are sublethally injured, such that in conditions of low pH, the cells are unable to repair the immediate damage, hence lowering their tolerance to the unfavorable pH and organic acids present in the juices (Garcia-Graells, Hauben, & Michiels, 1998).

For the inactivation of the enzymes, instead of using pressures higher than 600 MPa at room temperature, the use of lower pressures at increased temperatures is possible during HHP treatment. Processing temperature must be low to minimize undesirable color and flavor changes.

For the thermal treatment without pressure application, it was determined that the temperatures above 60 °C had significant effect on PPO inactivation in the apple juice and the inactivation showed diphasic behavior due to the presence of pressure-resistant isoenzyme (Oktay, Küfrevioğlu, Kocaçalışkan, & Şakiroğlu, 1995; Yemenicioğlu, Özkan, & Cemeroğlu, 1997). HHP treatment enabled the inactivation of PPO in apple juice (Figs. 1 and 2). The non-linearity of the curves was observed during HHP treatment. The activities of PPO dropped only slightly during the period of 30 min and the enzyme was increasingly inactivated after 30 min. A significant initial enhancement ($p < 0.05$) in activity for PPO in apple juice was observed at 450 MPa and 25 °C. This may arise from changes due to interactions

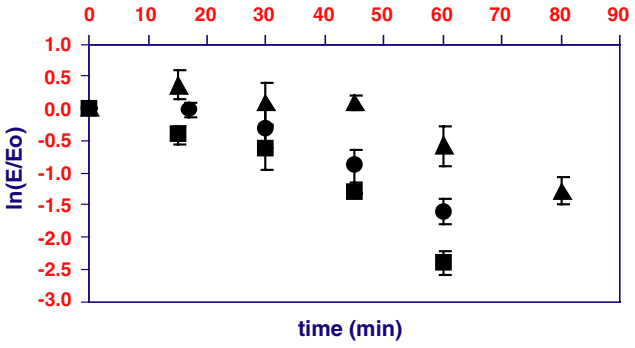

Fig. 1. Residual polyphenol oxidase (PPO) activities (E/E_0) in apple juice processed at 450 MPa (▲25 °C; ● 40 °C; ■50 °C) E: PPO activity after the treatment, E_0: initial PPO activity. Experiments and measurements were duplicated.

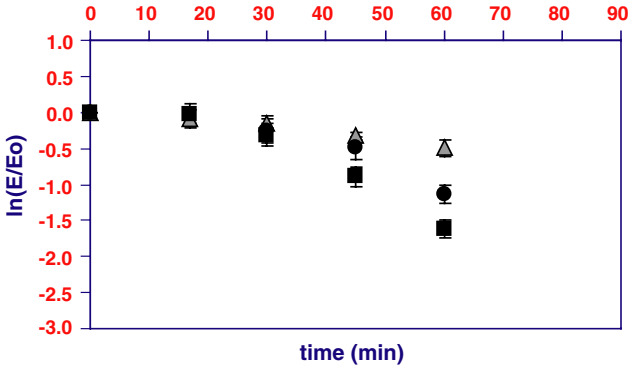

Fig. 2. Residual polyphenol oxidase (PPO) activities (E/E_0) in apple juice processed at 40 °C (▲ 0.1 MPa; ● 340 MPa; ■ 450 MPa) E: PPO activity after the treatment, E_0: initial PPO activity. Experiments and measurements were duplicated.

Table 3
Degree of browning in apple juice following 2 h storage at 25 °C

Treatment	Degree of browning[a]
Fresh (unprocessed)	3
0.1 MPa + 40 °C + 60 min	4
350 MPa + 40 °C + 60 min	2
450 MPa + 40 °C + 60 min	1
450 MPa + 25 °C + 60 min	2
450 MPa + 50 °C + 60 min	1

[a] 1: no browning, 2: slightly brown, 3: brown, 4: dark brown, 5: very dark brown (ΔA_{420} value of unprocessed fresh juice stored at 25 °C for 24 h was accepted as 5; very dark brown). Experiments and measurements were duplicated. Analysis of samples produced the same results.

with other constituents and from the release of membrane-bound enzymes and may relate to the pressure-activated latent form. The residual PPO activity in apple juice after treatment at 450 MPa and 50 °C for 60 min was obtained as 9 ± 2.25%.

Degree of browning after HHP treatment was related to the residual PPO activity in apple juice (Table 3). Although of high juice yield, Amasya apple has one of the highest rates of enzymatic browning among several apple cultivars. The apple juice when treated at 450 MPa with mild heat treatment, has a yellowish appearance and there was no further change in color (ΔA_{420}) during 2 h storage at room temperature. When the same samples were stored at 4 °C for 1 day, a significant change ($p < 0.05$) in color was not detected.

In the temperature range between 40 and 50 °C for a time period of 1 h without application of pressure, the activities of PE in orange juice dropped slightly. The residual activity was approximately 2% after heating at 90 °C for 1 min. Table 4 shows the inactivation of PE in orange juice at different combinations of pressure, temperature and time. The residual PE activity in orange juice after treatment at 450 MPa at 50 °C for 30 min was determined as approximately 7 ± 1.6%. It compares with 12 ± 0.2% at a treatment of 40 °C and 450 MPa for 60 min. Pressure-resistant isoenzymes were thought to be responsible for the final residual activity (Goodner et al., 1998). The inactivation is irreversible

Table 4
Residual PE activities in orange juice (pH = 3.76) processed at different conditions

P (MPa)	T (°C)	T (min)	Residual enzyme activity (%)[a]
350	40	30	24 ± 1.6
350	40	60	14 ± 2.3
350	50	30	13 ± 1.1
350	50	60	11 ± 0.1
450	40	30	20 ± 0.2
450	40	60	12 ± 0.2
450	50	30	7 ± 1.6
450	50	60	7 ± 0.2

[a] Experiments and measurements were duplicated.

Table 5
Cloud stability in orange juice following 1 and 2 week storage at 4 and 25 °C

Treatment	Cloud stability[a] (% transmission at 650 nm)			
	1 w storage		2 w storage	
	4 °C	25 °C	4 °C	25 °C
Fresh (unprocessed)	48 ± 4.2	83 ± 2.0	59 ± 3.1	86 ± 4.1
350 MPa + 25 °C + 30 min	33 ± 4.3	73 ± 1.2	39 ± 3.7	81 ± 2.8
450 MPa + 25 °C + 30 min	17 ± 3.3	39 ± 1.9	21 ± 0.6	49 ± 3.9
350 MPa + 40 °C + 30 min	23 ± 1.3	29 ± 4.2	27 ± 2.9	31 ± 3.5
450 MPa + 40 °C + 30 min	11 ± 4.1	17 ± 0.7	9 ± 4.1	15 ± 0.2

[a] Borderline value of 36% transmission at 650 nm (1 cm path length) is used as an upper limit for stable cloud in orange juice. Experiments and measurements were duplicated.

and the enzyme is not reactivated upon storage at 4 and 25 °C for 1 week. In citrus juices, an opaque nature is considered a desirable characteristic. Fresh orange juice usually loosens its cloud within a few days under refrigerated storage. Cloud loss is due to demethylated pectin interaction with calcium ions, causing a precipitation as a result a clear serum layer formation. Treated samples were stored at 4 and 25 °C for 2 weeks (Table 5). For quality control purposes a borderline value of 36% transmission at 650 nm (1 cm path length) is used as an upper limit for stable cloud in orange juice. During 2 week storage at 4 and 25 °C, a significant cloud loss ($p > 0.05$) was not observed in the orange juice treated at 350 MPa and 40 °C for 30 min. 450 MPa, 25 °C and 30 min combination provided cloud stability only in orange juice stored at 4 °C. Ascorbic acid is an important nutrient in orange juice and heat treatment has a great effect on the reduction of ascorbic acid. After HHP processing at the selected range of pressure (350–450 MPa), temperature (25 °C) and time (30 min) combinations, no significant differences ($p > 0.05$) in ascorbic acid content of the orange juices were detected if compared with that of the fresh juice (the amount of ascorbic acid in control: 45.7 mg/100 ml). Only, when the processing temperature was increased to 40 °C at 450 MPa for a holding time of 30 min, the ascorbic acid loss was determined as 7 ± 1.6%. Further studies should be carried on the effect of HHP on vitamins in fruit juices.

This work has shown that commercially practicable pressure processes can be used to inactivate even the most pressure-resistant strains of *S. aureus*, *E. coli* O157:H7 and *S.* Enteritidis. The use of HHP (350 MPa) at 40 °C could be considered for treating the fruit juices studied to improve microbial kill, with respect to the pressure-resistant strains of pathogens studied. High pressure processing constitutes an effective technology to inactivate the enzymes in fruit juices. Pressures higher than 400 MPa can be combined with mild heat (<50 °C) to accelerate enzyme inactivation. The processing time needed to inactivate enzymes is much longer than that needed to kill bacteria in acid juices. This situation is similar to heat treatment: higher temperature is required to inactivate bacterial spores and enzymes than to achieve commercial sterility. Therefore, during the selection of processing conditions (pressure–temperature–time) for commercial applications, this information may be of value if pressure combined with mild heating in terms of the selection of inactivation target.

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