EFFECT OF UHP ON ENZYME, MICROORGANISM AND FLAVOR IN CANTALOUPE (*CUCUMIS MELO* L.) JUICE

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ABSTRACT

In this study, we investigated the change of the flavor, enzyme activities and microorganism survival in cantaloupe (*Cucumis melo* L.) juice after UHP treatments, and found the corresponding kinetic models. After the treatment at 500 MPa for 20 min, the pressurized juice can reach the safety standards, i.e., below 100 cfu/100 mL in concentration stipulated for beverage by Chinese national standard; and the activities of POD, PPO and LOX in the juice decreased to about 78, 9 and 5%, respectively. Sensory evaluation indicated that no significant change was observed after UHP treatments. The above findings indicated that UHP treatment is a promising way to process the cantaloupe juice.

PRACTICAL APPLICATION

Cantaloupe is a favorable fruit with unique aroma produced around the world. However, because there is no effective deep-processing, a lot of cantaloupes rot away in the farmland every year. The production of freshly squashed juice is a best way to raise the merchandise rate of cantaloupe and to

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prolong its industrial chain. However, freshly squashed cantaloupe juice has a very short shelf life because it will quickly succumb to microorganism attack if nothing is done. The sterilization of its juice is a difficult issue because the juice is heat sensitive. Therefore, nonthermal processing is desirable. UHP technology used in this study was proven to be a promising way to maintain the flavor and to inactivate enzymes and microorganisms in the cantaloupe juice.

**INTRODUCTION**

Cantaloupe (*Cucumis melon* var. recticulatus. Hami melon) is a favorable fruit with unique aroma produced in Sinkiang Uigur Autonomous Region, P. R. China. However, because there is no effective deep processing, only a little portion is sold at a lower price on-site, while a lot of cantaloupes rot away in the farmland every year. Its merchandise rate is only about 50%, which has strictly bottlenecked the development of the cantaloupe industry (SBSUAR 2002). The production of freshly squashed juice is the best way to raise the merchandise rate of cantaloupe and to prolong its industrial chain.

As one of the most important indexes in quality evaluation, the unique flavor of cantaloupe not only distinguishes it from other varieties of melons but also marks it in perfect maturity. However, freshly squashed cantaloupe juice has a very short shelf life because it will quickly succumb to microorganism attack if nothing is done. The sterilization of its juice is a difficult issue because the juice is heat sensitive. Heating for only few minutes will make the juice produce cooked off-odor, in addition to the damage to vitamins and nutrient content (Hayashi and Balney 1996). In contrast, foodstuff treated with UHP retains the flavor (Cheftel 1995), the color, the taste of natural foodstuffs (Butz *et al.* 1994) and the natural properties of the products (Ashie and Simpson 1996; Ennen 2001). Therefore, UHP treatment could be a promising way to solve the problem.

UHP treatment has different influences on the quality of different foodstuffs. When the high-pressure treatment is used for microorganism and enzyme inactivation in the fruit juice process, there occur some changes in aroma or flavor of fruit juice (Kazeniac *et al.* 1975; Porretta *et al.* 1995; Zabetakis *et al.* 2000a,b). Hendrickx *et al.* (1998) reviewed that the POD activity in strawberry cannot be reduced until the 15 min under 400 MPa at ambient temperature, and it increased after UHP treatment at from 32°C to 60°C. The PPO in mushroom or potato has pressure resistibility, and cannot be inactivated until at 800, even 900 MPa. PPO in different fruits, such as grapes, strawberries, apricots and apples, starts to be inactivated at different pressures. The UHP could change the permeability of the microorganism cell membrane,
break the microorganism cell membrane and make the component in the microorganism body leak, and at last induce enzyme inactivation, protein quality change and directly or indirectly DNA damage, so that the microorganism suffers metabolic error or metabolic function loss (Lucore et al. 2000; Richard 2000). However, so far, little information is available on cantaloupe juice treated with UHP, especially the kinetic models on microorganism and enzyme inactivation, which are practically significant for design and production of cantaloupe juice processing, for food safety and for database establishment of food information technology. The purpose of this study was to examine the influence of high-pressure treatment on flavor, enzyme activity, survival ratio of *Escherichia coli* (*E. coli*), *Bacillus subtilis* bacteria and total colony in cantaloupe juice, and to find the corresponding kinetic models. “Golden Empress” cantaloupe is chosen because of its unique flavor.

**MATERIALS AND METHODS**

**Experimental Apparatus**

The hyperbaric apparatus used in the experiment was designed and produced by the Chinese Academy of Agricultural Mechanization Sciences (Beijing, P. R. China) with maximum working pressure of 600 MPa, and oil was used in the apparatus as the pressure fluid. Other apparatus used were a juice extractor (HL-60), a digital pH meter (pH 38), a portable Brix refractometer (WAY-2S, ABBE), a homogenizer with maximal working pressure of 60 MPa/maximal flow of 60 L/h and a centrifuge.

**Samples**

The experimental samples were Golden Empress cantaloupe (*C. melon. var. recticulatus. Hami melon*), a variety planted in Sinkiang Uigur Autonomous Region, P. R. China. Golden Empress melon samples were fetched randomly from a heap (10 × 5 × 5 boxes). All the selected melons were fully ripe without any quality deterioration or decay.

**Preparation of the Melon Juice**

At first, the fresh melon was washed and sanitized with bleaching powder (calcium hypochlorite) solution of 100 mg/kg in a bucket for 15 min and then washed with clear tap water. Next, it was peeled manually, and then the pedicel section and calyx section were cut away. Thirdly, seeds and its circumambient section were removed and cleared off. Fourthly, the melon was cut into pieces, put into the juice extractor, and squashed for less than 30 s. Fifthly, the juice
was homogenized with the homogenizer at 40 MPa. Lastly, the juice was vacuum-sealed into sterile laminate bags and directly used for the UHP treatments. Each bag has a capacity of 100 mL. Each sealing was carried out at 100°C under 80% vacuum degree within 4 s. The juice has the soluble solid content of 12 Brix with pH value ranging from 5.6 to 5.8.

**UHP Treatment of Samples**

The samples were placed in a 3-liter compression chamber and subject to hydrostatic pressures of 500 MPa for 20 min at the ambient temperature (22°C) with pressure-increasing rate of 100 MPa per min. The pressure-releasing time was about 12 s; the oil temperature in the pressure cabin was between 20 and 22°C; and the samples after the pressure treatment were kept at 2–10°C in refrigerator for further determination, and all determinations were conducted within 2 h after the end of pressure treatment.

**Determination of POD Activity**

After centrifugation with 1,960 × g for 20 min at 4°C the supernatant liquid of the melon juice treated with UHP was used as the enzyme extracting solution to be detected. Guaiacol method was used to detect the POD (Chance and Maehly 1967). The enzyme activity was expressed with optical density (OD/min) detected after reaction for 1 min using 1 mL of enzyme extracting solution. One unit of enzyme activity was defined as $1 \times 10^{-3}$ ΔOD/min.

**Determination of Lipoxidase Activity**

Preparation of substrate was performed with the improved Surrey method (Surrey 1964), and the LOX activity was measured with Axelrod method (Axelrod et al. 1981). One unit of enzyme activity was defined as $1 \times 10^{-3}$ ΔOD/min.

**Determination of PPO Activity**

One milliliter of catechol solution with concentration of 0.1 mol/L was added in 3.9 mL of disodium hydrogen phosphate and citric acid buffer. The solution was heated in a water bath at 30°C, and then 0.1 mL of the enzyme extraction (the supernatant solution) was put into the solution. Absorbance at 420 nm was monitored continuously for 5 min per 20 s at 30°C under the spectrometer, and the activity was determined from the slope of the linear portion of the curve. One unit of enzyme activity was defined as $1 \times 10^{-3}$ ΔOD/min.
Isolation and Culture of Microorganism

The dominant microorganisms in the melon juice after UHP treatment were isolated and cultivated with media (10 g glucose, 3 g beef extract, 5 g sodium chloride, 5 g peptone, 15 g agar, 1,000 mL water) adjusted into pH 7.2, and sterilized for 30 min at 121°C according to the standard methods (Zhou 1997). The plate count method stipulated by GB4789.2-94 (Liu 1994) was used to calculate the amount of bacteria (Song et al. 2006).

Preparation of *Bacillus subtilis* Suspension

The dominant species of separated spore was *Bacillus subtilis*, identified by Genetic Lab in China Agricultural University and coded as *Bacillus subtilis* UHP1. The species were inoculated onto the Petri dish of culture medium A (as mentioned above), cultivated at 28°C for 48 h, then scrubbed down with a sterile glass shovel and washed twice with the sterile normal saline. Subsequently, microbial flora was poured into a sterile test tube and broken up with an oscillator; and then sub-packaged into several sterile test tubes and heated in the water at 80°C for 15 min. After the vegetative cells were killed, the spore water suspension was adjusted to about $10^7$ cfu/mL in concentration with the plate count method. The spore suspension was sub-packaged into the sterile laminate bags for the UHP treatment.

Preparation of *E. coli* Suspension

The *E. coli* strain was *Escherichia coli* (Migula) Castellani et Chalmers, 1.9, which was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (China). The *E. coli* strain was transferred and incubated in nutrient agar slant culture-medium for 24 h at 37°C, and then microbial flora were washed from the slant with sterile phosphate buffer (pH 6.86), collected and shook for uniform in a sterile test tube to become a bacteria suspension. The suspension was adjusted to about $10^7$ cfu/mL levels with the plate count method wherein the diluent was the phosphate buffer; and then it was sub-packaged into sterile laminate bags and stored at 4°C before and after the UHP treatment. After the UHP treatment, the surviving viable *E. coli* in the samples was incubated again in the nutrient agar culture medium and counted according to the plate count method stipulated by GB4789.2-94.

Difference Test

It is a procedure for determining whether there exists a perceptible sensory difference or a similarity between samples of two products concerning the intensity of a sensory attribute. This test is sometimes also referred to as a paired comparison test and duo-trio test. It is effective for determining whether
Therefore, the difference test method was adopted to identify the aroma difference between Golden Empress melon juice sample A and B treated, respectively, with 400 MPa and 500 MPa for 20 min as well as sample C untreated with UHP. Every two samples were grouped into a pair and the pairs, including their coding, are listed in Table 1. In the paired comparison test, the sample pairs and their repetitions in the order as in Table 1 were served to each of 10 testers. Each tester judged four pairs and recorded whether the aromas of two samples in each pair were different or not. Therefore, the number of trials was 40. Similarly, in the duo-trio test, a reference sample was firstly served to a tester, after evaluation the reference sample was removed away from the tester and then two samples as a pair including the reference sample were served to the tester for evaluation. Each of 10 testers judged four pairs and recorded whether the aromas of two samples in each pair were different or not. Therefore, there were 40 trials in each duo-trio test. According to sensory evaluation results, the statistical analysis and judgment were performed (Stone and Sidel 1985; Li et al. 1990).

### Data Detection and Processing

All measurements in the experiment were performed in triplicate. All the diagrams and regression equations were processed with the statistics software Origin Pro7.5. The significance level was less than 5% ($P < 0.05$) in all regression equations. The first-order exponential attenuation model used was expressed as follows:

$$Y = A_1 \times \exp\left(-\frac{X}{t_1}\right) + y_0$$  \hspace{1cm} (1)

where $Y$ stands for the residual activity of enzyme (OD/min), or, the logarithmic value of residual microorganisms after UHP; $X$ represents the pressurized time (min); and $A_1$, $t_1$ and $y_0$ are constants in the model derived from the statistical regression analysis.
RESULTS AND DISCUSSION

Inactivation Effects of UHP Treatment on *E. coli*, *Bacillus subtilis* and Total Colony

Total colony in the juice can be almost completely killed after the treatment at 500 MPa for 15 min (Fig. 1), reduced from $(3.89 \pm 0.26) \times 10^4 \text{ cfu/mL}$ to $(20.40 \pm 0.04) \text{ cfu/mL}$ in concentration, which was below the lower limit of detection (LLD), 100 cfu/100 mL, stipulated for beverages by Chinese national standard. The concentration of *E. coli* decreased from $(2.08 \pm 1.10) \times 10^7 \text{ cfu/mL}$ to $(1.78 \pm 0.53) \times 10^5 \text{ cfu/mL}$, namely, an extent of five logarithmic phases, within 8 min at 500 MPa, which met the nonthermal bactericidal hygienic standard stipulated by the American FDA (2001). This observation was similar to the previous results. For instance, Alpas *et al.* (1999) investigated the pressure resistance of six *E. coli O157:H7* strains at 345 MPa for 5–15 min at 25 and 50°C. The higher temperature level yielded more than eight log-cycles reduction within 5 min.

The *Bacillus subtilis* treated at 500 MPa for 20 min decreased from $(2.95 \pm 0.55) \times 10^7 \text{ cfu/mL}$ to $(1.69 \pm 0.54) \times 10^5 \text{ cfu/mL}$ in concentration, namely, a 3.25-log reduction, which indicated that the spore bacteria have higher-pressure resistibility as compared to other bacteria. The reason was, after sporulation, the resulting organism is extremely resistant to external attack by physical or chemical means.

![FIG. 1. RELATIONS BETWEEN PRESSURIZED TIME AND MICROORGANISM](image-url)
On the other hand, the initial amount of microorganism in raw material of the melon is an important factor for raising the bactericidal effect. The impact of the initial microbial population on spore inactivation has been studied by Furukawa et al. (2003). It was observed that the inactivation rate decreased as the initial bacterial concentration increased. They suggested that in practical application of hydrostatic pressure treatments, the initial concentration of bacteria should be as low as possible.

It is helpful to control and reduce the initial concentration of microorganism in the melon juice to the utmost extent. The initial colony population in raw material of the melon is mainly subject to implantation, postharvest treatment, and process conditions in sorting, cleaning and disinfecting, peeling, cutting, pulping and storage time etc. According to our experimental results, the initial colony population in the raw material after washing and disinfecting can be limited within $10^4$ cfu/mL, which could warrant the safety of the melon juice after the UHP treatment at 500 MPa. Namely, the colony population could reach a 5-log reduction demanded by the FDA hygienic standard, or, below 100 cfu/100 mL in concentration stipulated for beverages by Chinese national standard. Therefore, UHP treatment is a promising method to keep quality of the melon juice.

### Sterilization Kinetic Models of UHP Treatment

The regression equation between the pressurized time and the logarithmic values of surviving total colony in the juice, such as mould, cocci and non-bacillus, or surviving *E. coli*, or surviving *Bacillus subtilis* well followed the first-order exponential attenuation model. Corresponding parameters in Eq. (1), $A_1$, $t_1$ and $y_0$, are illustrated in Table 2.

The square correlation coefficients were 0.931, 0.975 and 0.9673 respectively (Table 2), which meant that the first-order exponential attenuation model

<table>
<thead>
<tr>
<th>Bacteria or enzyme</th>
<th>Parameters</th>
<th>$y_0$</th>
<th>$A_1$</th>
<th>$t_1$</th>
<th>$R^2$</th>
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<tr>
<td>Spore bacteria</td>
<td></td>
<td>3.3447</td>
<td>4.0045</td>
<td>17.8988</td>
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<tr>
<td>Total colony</td>
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<td>1.4790</td>
<td>3.0842</td>
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<tr>
<td><em>E. coli</em></td>
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<td>1.8310</td>
<td>5.3896</td>
<td>3.6596</td>
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</tr>
<tr>
<td>Peroxidase</td>
<td></td>
<td>$-2,489.8367$</td>
<td>2,490.5481</td>
<td>240,788.9902</td>
<td>1</td>
</tr>
<tr>
<td>Polyphenoloxidase</td>
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<td>$-0.0017$</td>
<td>0.4637</td>
<td>6.9740</td>
<td>0.9146</td>
</tr>
<tr>
<td>Lipoxygenase</td>
<td></td>
<td>0.0831</td>
<td>0.5668</td>
<td>0.0367</td>
<td>0.9391</td>
</tr>
</tbody>
</table>
could well describe the UHP treatment change histories of residual spore bacteria, total colony and *E. coli*. The values of characteristic constant, t1, in the models indicated that, when the concentration log of residual spore bacteria, or total colony, or *E. coli* decreased by 63% of the value of parameter A1, the corresponding inactivation times were about 17.9, 2.9 and 3.7 min, respectively. Therefore, it was another expression to say that *E. coli* and the total colony in the juice were liable to inactivation by UHP treatment, and *Bacillus subtilis* was the most difficult to be inactivated.

**Enzyme Inactivation Effects of UHP Treatment**

The enzyme activity of POD, PPO and LOX in the melon juice at 500 MPa was subject to the pressurized time (Fig. 2). The enzyme activity of POD decreased slightly during the first 8 min, and decreased by (18.99 ± 1.63)% when the pressurized time was up to 10 min. Since then, it had been almost unchanged. Its survival ratio was (77.91 ± 0.89)% at the end of UHP treatment. The enzyme activity of PPO was almost unchanged in the first 3 min, and then decreased quickly by (43.25 ± 5.55)%, (69.69 ± 2.41)% and (90.94 ± 1.47)% when the pressurized time was up to 5, 8 and 10 min, respectively. Its survival ratio was (8.78 ± 0.65)% at the end. LOX was liable to inactivation because it only took 3 min to decrease by (92.88 ± 1.96)%. Between the 5th and 8th min, however, the enzyme activity of LOX was

![Graph showing the relations of pressurized time and enzyme activity](image-url)
almost unchanged, and then bounced back a little from the 10th to 15th min. Finally, at the 20th min its survival ratio was \((5.42 \pm 1.02)\%\).

Conclusively, the three enzymes in the melon juice still maintained their activities more or less at 500 MPa for 20 min, of which the survival ratio of POD was the largest one, followed by PPO and LOX in sequence. This observation was in agreement with the report by Seyderhelm et al. (1996). They ranked the enzymes according to their pressure induced inactivation in the following order (from low to high): LOX, lactoperoxidase, pectinesterase, lipase, phosphatase catalase, PPO and POD by considering distinct conditions within a pressure range of 0.1–900 MPa.

**UHP Kinetic Models of Enzyme Inactivation**

The kinetic curves of POD, PPO and LOX activities (OD/min) met the first-order exponential attenuation model, and their model parameters were listed in Table 2. All the models can be well used for prediction, suggested by high correlation coefficients, i.e., the values of \(R^2\) were more than 0.91. The values of \(t_1\) in Table 2 indicated that, when the log values of residual activity of POD, PPO and LOX decreased by 63% of the values of parameter \(A_1\) (2,490.5481, 0.4637 and 0.5668), the corresponding inactivation times were about 240,789, 6.974 and 0.0367 min, respectively. Therefore, the same conclusion can be expressed by theses parameters, i.e., the LOX was susceptible to inactivation with UHP, and POD was the most difficult to be inactivated.

**Aroma Difference Evaluation of the Melon Juice Treated with UHP**

According to the difference test method, 10 testers with sensory evaluation experience were selected. Each tester evaluated four groups of coded pair samples. Forty tests were performed, and the results were listed in Table 3. Most difference tests are one tailed; the tail refers to a segment of the distribution curve (i.e., the tail end). The paired comparison test and the duo-trio test used in our experiment are one tailed because the “no-decision” option was not permitted so that there was a single correct outcome in our difference test. Therefore, according to the one-tailed probability table of difference test, when the number of trials was 40, and the difference number were respectively 23, 21 and 25 (Table 3), their probability levels were: \(P_1 = 0.215 > 0.05\), \(P_2 = 0.437 > 0.05\) and \(P_3 = 0.077 > 0.05\), respectively (Stone and Sidel 1985; Li et al. 1990). Therefore, at the significant level of 5%, there was neither significant difference between the melon juice sample A, treated with 400 MPa, and the sample B, 500 MPa, nor between the sample C untreated and the sample A, or the sample B.
CONCLUSION

Sensory evaluation indicated that there was no significant aromatic difference between UHP treated and untreated melon juice samples, as well as between the samples treated at 400 and 500 MPa. The *Bacillus subtilis* had higher-pressure resistibility in the melon juice as compared to other bacteria. The initial colony population in the raw material after washing and disinfecting treatment can be limited within $10^4 \text{ cfu/mL}$. After the treatment at 500 MPa for 15 min, total colony can be almost completely killed in the melon juice; the concentration of *E. coli* as safety index decreased by 5-log cycles within 8 min at 500 MPa, which met the nonthermal bactericidal hygienic standard stipulated by the American FDA. The logarithmic values of surviving spore, surviving *E. coli* and surviving total colony in the juice well followed the first-order exponential attenuation model with square correction coefficients of more than 0.93. Three enzymes in the melon juice still maintained their activities at 500 MPa for 20 min, and the survival ratio of POD, PPO and LOX was about 78, 9, and 5%, respectively. Surviving activities of POD, PPO and LOX followed well the first-order exponential attenuation model. Although it cannot completely inactivate some enzymes such as POD and PPO, UHP is a recommendable and promising method to keep flavor profile and safety of the melon juice.

<table>
<thead>
<tr>
<th>Panel</th>
<th>A versus B</th>
<th>A versus C</th>
<th>B versus C</th>
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<tr>
<td></td>
<td>Different</td>
<td>Same</td>
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<tr>
<td>1</td>
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<td>1</td>
<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>17</td>
<td>21</td>
</tr>
</tbody>
</table>

C represents the fresh juice untreated with UHP; A represents the sample treated with 400 MPa; and B is the sample treated with 500 MPa.

**TABLE 3. RESULTS OF DIFFERENCE TEST FOR PRESSURIZED FRESH GOLDEN EMPRESS MELON JUICE**
NOMENCLATURE

UHP Ultra high pressure
PPO Polyphenoloxidase
POD Peroxidase
LOX Lipoxygenase

ACKNOWLEDGMENT

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REFERENCES


