Influence of controlled atmosphere on thermal inactivation of *Escherichia coli* ATCC 25922 in almond powder

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ABSTRACT

Heat controlled atmosphere (CA) treatments hold potential to pasteurize *Salmonella enteritidis* PT 30 in almonds. Nonpathogenic *Escherichia coli* ATCC 25922 was used as a surrogate species of pathogenic *Salmonella* for validation of thermal pasteurization to meet critical safety requirements. A controlled atmosphere/heating block system (CA-HBS) was used to rapidly determine thermal inactivation of *E. coli* ATCC 25922. *D*- and *z*-values of *E. coli* ATCC 25922 inoculated in almond powder were determined at four temperatures between 65 °C and 80 °C under different gas concentrations and heating rates. The results showed that *D*- and *z*-values of *E. coli* under CA treatment were significantly ($P < 0.05$) lower than those under regular atmosphere (RA) treatment at 4 given temperatures. Relatively higher CO_2 concentrations (20%) and lower O_2 concentrations (2%) were more effective to reduce thermal inactivation time. There were no significant differences in *D*-values of *E. coli* when heating rates were above 1 °C/min both in RA and CA treatments. But *D*-values significantly ($P < 0.05$) increased under RA treatment and decreased under CA treatment at lower heating rates. Combination of rapid heat and CA treatments could be a promising method for thermal inactivation of *S. enteritidis* PT 30 in almond powder.

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1. Introduction

Almond is a popular tree nut and offers many benefits to human health (Li et al., 2016). The total production of almonds in USA, Australia, Spain, Italy, China and Turkey, was approximately 2.918 million tons in 2013 (FAOSTAT, 2013). There is a potential food safety problem with various microbial contaminations caused by infected soil, water, and air during harvest, drying, transport, and processing periods. In recent years, concerns on frequent outbreaks in low-moisture foods have increased (Kimber et al., 2012), because foodborne pathogens in low moisture food can survive for long time, resulting in many potential food safety issues once the external conditions are suitable for their rapid propagations (Ledet Muller et al., 2007). Many cases of *S. enteritidis* foodborne infection indicate the importance of *Salmonellosis* as a major public health problem (Icgen et al., 2002). Especially in 2000 and 2004, the outbreak of *Salmonellosis* associated with raw almonds caused 168 illnesses (Isaacs et al., 2005; Ledet Muller et al., 2007). Therefore, it

is important to reduce pathogens in raw almonds into an acceptable level prior to export as mandated by the US Department of Agriculture (USDA) (Niemira, 2012).

Thermal treatments are considered to be one of the most effective methods for elimination of *Salmonella* in food processing and preparation (Jarvis et al., 2016). Thermal pasteurizations of almonds have been studied or are under development using many methods, including hot water (Harris et al., 2012), hot oil (Du et al., 2010), infrared radiation (Bingol et al., 2011; Yang et al., 2010), steam (Chang et al., 2010), and radio frequency heating (Gao et al., 2011; Li et al., 2017; Marra et al., 2009). The pathogens are thermally resistant in low moisture food, which requires long time at high temperature to achieve the safe level, resulting in potential negative effects on product quality. For example, exposed *S. enteritidis* PT 30 to hot water at 88 °C for 2 min achieved 5-log reduction, but led to the almond skin loosening (Harris et al., 2012). After 30 s exposed to hot oil at 127 °C, 3.6-log reduction was observed, but hot oil treatment is only used for roasted almonds not for raw almonds (Du et al., 2010). Combination of sequential infrared radiation treatment with traditional hot air (SIRHA) roasting at 140 °C for 18 min achieved 4-log reduction of *Pediococcus* sp. NRRLB-2354, which was used as a surrogate for

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S. enteriditis PT 30, but the almond quality was not assessed after SIRHA treatments (Bingol et al., 2011; Yang et al., 2010). Combining heat with controlled atmosphere (CA) has been shown to reduce the thermo-tolerance of pathogens and maintain the product quality (Kawachi et al., 2015) and could be a potential method for pasteurizing almonds.

A lot of related researches have been carried out for pathogen control in foods using combined heat and CA treatments. Modified atmosphere packaging (MAP) may reduce pathogen growth in ground beef (Brooks et al., 2008), cheese (Govaris et al., 2011; Pintado and Malcata, 2000), ground chicken breast meat (Irkin and Esmer, 2010), hen table eggs (Pasquali et al., 2012), ready-to-eat (RTE) shrimp (Rutherford et al., 2007), and fresh common carp (Hudecova et al., 2010). Modified atmosphere (CO_2: O_2: N_2 = 30%: 5%: 65%) has been used to inhibit the growth of total air-borne microorganisms and E. coli in RTE products stored at 25 °C (Chen et al., 2003). Compressed oxygen and carbon dioxide are used to reduce the microbial populations in powdered white and black pepper (Kawachi et al., 2015). It's important to determine the thermal inactivation kinetics for pathogens in developing effective pasteurization protocols using combined heat and CA treatments.

Because of critical safety requirements, S. enteritidis PT 30 cannot be directly used to conduct thermal pasteurization validation tests in food processing plants. Using nonpathogenic E. coli ATCC 25922 as a surrogate species of pathogenic Salmonella for validation of thermal pasteurization is an alternative way. Thermal resistance of E. coli ATCC 25922 in almond is stronger than that of S. enteritidis PT 30 under the same treatment conditions (Eblen et al., 2005; Li et al., 2017.), suggesting that E. coli ATCC 25922 could be used as a surrogate of S. enteritidis PT 30 for validating thermal pasteurization processes.

Choosing an appropriate test method is important to accurately obtain the bacteria's D-(Decimal reduction time at a given heating temperature) and z-(Temperature changes needed for 90% reduction in D-values) values. Thermal death time test is a common method to determine the heat resistance of bacteria (Buchner et al., 2012). Heating rate in samples cannot be controlled in water or oil bath methods due to difference of sample's thermal properties, although it is a major factor to influence the thermo-tolerance of bacteria (Kou et al., 2016). A controlled atmosphere/heating block system (CA-HBS) has been successfully used to rapidly determine thermal death kinetics of insect pests under various heating rates (Li et al., 2015a,b). The basic requirements of precisely controlled sample temperatures, stability and accuracy of gas concentrations need to be evaluated before using the CA-HBS to quickly assess the thermal inactivation of E. coli ATCC 25922.

The objectives of this study were to 1) verify stability of gas tightness, gas concentrations and temperatures of CA-HBS under RA and CA treatments, 2) determine D- and z-values for E. coli ATCC 25922 in almond powder under given CA and RA conditions using the CA-HBS, 3) determine effects of specific CO_2 and O_2 concentrations on thermal resistance of E. coli, and 4) explore the effect of heating rates on D -value of E. coli ATCC 25922 under CA and RA treatments.

2. Materials and methods

2.1. Preparation of materials

Almonds (Nonpareil) were purchased from Paramount Farming Company (Modesto, CA, USA). Almond kernels were ground in a blender (FLB-100, 220 V, 50—300 mesh, Philip Bo Food Machinery Corp, Shanghai, China) and then passed through a 18 mesh/inch sieve to obtain powder (≤1.0 mm) samples. The original moisture content of almonds powder was 5.46% wet base (w.b.), which was determined by oven methods according to the AOAC standards (AOAC, 2002). The 6% w.b. samples were obtained by adding 0.23 g distilled water to 40 g of flour sample at the initial moisture content. The adjusted samples were conditioned in closed containers under refrigeration (4 °C) for at least 2 days before use.

E. coli ATCC 25922 strains were obtained from the College of Food Science and Engineering, Northwest A&F University (Yangling, China). Stock cultures were made in Luria-Bertani broth (LB: Beijing Land Bridge, Beijing, China) and stored at −20 °C with 15% (vol/vol) glycerol. Stock cultures were allowed to thaw at room temperature (25 °C) for 5 min (Li et al., 2017). A loopful was then streaked onto LB agar and incubated at 37 °C for 24 h. Single colony was streaked onto LB agar and incubated at 37 °C for 24 h. Then a signal colony was transferred into 30 ml LB broth and incubated for 24 h at 37 °C. Finally 3 ml bacterial suspension was transferred to 300 ml LB broth and incubated again at 37 °C for 24 h. The cell population was adjusted to a level of 10^{10} CFU/ml. 2 g 6% w.b. almond powder was put in a 5×5 cm nylon mesh bag (300 mesh/inch). 25 μl cell population of 10^{10} CFU/ml E. coli ATCC 25922 was inoculated and then shaken evenly 1 h before testing. Finally, samples were sealed into plastic bags (80 mm × 120 mm) and stored no more than 2 h at 4 ± 0.5 °C before testing to avoid water entering mesh bags during cooling processes and bacteria contamination in the air to influence enumeration of E. coli.

2.2. Verifying stability of the CA-HBS

2.2.1. Gas tightness of the CA-HBS system

Unstable gas concentrations could be caused by possible gas leaks from the gas delivery lines and the seal situations between the top and bottom heating blocks (Li et al., 2015a,b). The CO_2 and O_2 flow meters were set at 500 ml/min and 50 ml/min, respectively (Fig. 1). The N_2 flow meter was adjusted to 350, 450 and 550 ml/min to obtain flow rates of 900, 1000, and 1100 ml/min, respectively. Relative leakage was obtained based on the readings of the fourth and fifth flow meters. The values of all flow meters were recorded once every 5 min throughout each test, which was replicated three times.

2.2.2. Stability of O_2 and CO_2 concentrations

Since 0—25% range of O_2/CO_2 was used in CA treatment, the stability of gas concentrations in the chamber was analyzed at three set point levels: 1% O_2/25% CO_2, 5% O_2/10% CO_2, and 20% O_2/1% CO_2. The CA concentration-time profile was obtained by sampling every 5 min over 60 min. Gas samples were also taken after exited the chamber to ensure that gas concentrations within the gas chamber remained stable. After the stable set point was reached, the gas samples both for entering and exiting the chamber were taken every 3 min for 30 min. Each gas level was replicated three times. The average values and standard deviations were calculated based on three replicates for each test.

2.2.3. Temperature stability

As shown in Fig. 2 a, three representative positions (P_1, P_2, and P_3) on the bottom block surface were selected to observe temperature variations. Surface thermo-couples (SA1-T, Omega Engineering Ltd, CT, USA) and a data acquisition system (CR-1000, Campbell Scientific, Inc, Logan, Utah, USA) were used for temperature measurements. The temperature variations were analyzed under RA and CA treatments, respectively.

2.2.4. Changes in moisture content (MC) and water activity (A_w)

Thermal inactivation of pathogens can be influenced by moisture content (MC) (Nascimento et al., 2012; Silva and Gibbs, 2012) and water activity A_w (Villa-Rojas et al., 2013) in almonds. The
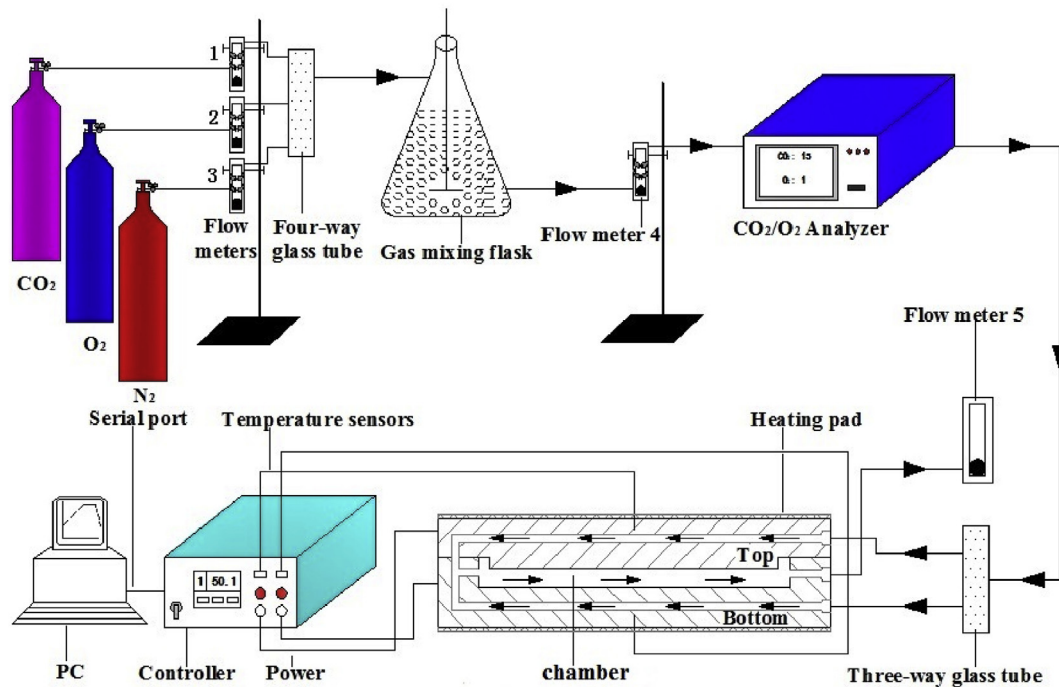
Fig. 1. Diagram of controlled atmosphere/heating block systems, including gas cylinders, flow meters, gas mixing flask, O_2/CO_2 analyzer, and heating blocks with computer controlling and monitoring (adapted from Li et al., 2015a,b).

changes of MC and A_w were necessary to be considered during the test. The MC was determined according to the AOAC standards (AOAC, 2002). A_w of almond shell and kernel was determined by Aqua Lab water activity meter (Model 4TE, Decagon Devices, Inc, Pullman, WA, USA). About 5—6 g of almond flour samples were placed into a sample cup and then in the water activity meter to measure A_w under ambient temperature (25 °C).

2.3. Treatments

2.3.1. Temperature-time history curve measurements

To investigate effects of the modified atmosphere on the thermal inactivation of *E. coli*, a 5 cm × 5 cm, 300 mesh bag was used to hold the samples. A pre-calibrated Type-T thermocouple (TMQSS-020-6, Omega Engineering Ltd., CT, USA) was installed in the center of the sample and the bottom plate to measure temperatures, which were recorded every second by the data logger. The temperature-time history was measured for each run using the non-inoculated sample, and recorded in a computer. The recorded temperature-time history curve obtained was used to determine the come-up time and time zero for each of four target temperatures.

2.3.2. Heating treatments

25 µl of *E. coli* ATCC 25922 bacterial suspensions with 10^{10} CFU/ml cell populations were placed inside 2 g almond powder packing in the mesh bag as a test sample. Five samples were placed in five representative points on the bottom plate. As controls, another sample was treated in the same way for the longest holding time without heating. The temperatures were controlled at 65, 70, 75 or 80 °C with a heating rate of 5 °C/min. The come-up time for the samples to reach within 0.5 °C of each set-point temperature was determined and used as time zero to provide close-to-ideal isothermal conditions. Samples were removed at five different

time intervals, depending on the temperature, to achieve at least a 5-log reduction. After holding end, the mesh bags were immediately sealed in the plastic bags and immersed in an ice-water bath (about 4 °C, for at least 2 min) until further analysis was performed. Based on previous test results, a treatment of 75 °C was selected to compare the effect of heating rates of 0.1, 0.5, 1, 5 and 10 °C/min under both RA and CA conditions (2% O_2/20% CO_2) on *D*- and *z*-values of *E. coli*.

2.3.3. Gas treatments

0-25% range of O_2/CO_2 concentration was used in CA treatment to determine *D*- and *z*-values of *E. coli*. O_2 concentrations were set at 0, 2, 5, 10, 15, and 21% with a flow rate of 950—1000 ml/min under no CO_2 conditions. CO_2 concentrations were set at 0, 5, 10, 15, 20, and 25% with a flow rate of 1000—1100 ml/min under 2% O_2 concentration. Mean values and standard deviations of *D*- and *z*-values were calculated over three replicates.

2.4. Microbiological inactivation kinetics and enumeration

Plotting log *D*-values against temperatures often reveals a linear relationship, commonly referred to as the thermal death time curve (Chung et al., 2007). The following equation was used to describe the survival curve:

$$\frac{N}{N_0} = 10^{\frac{-t}{D}} \tag{1}$$

Where *N* is the surviving populations of *E. coli* ATCC 25922 at a given time (CFU/g), N_0 is the initial population of *E. coli* ATCC 25922 (CFU/g), *t* is the heating time (min) and *D*-value was obtained by the reciprocal of the microbial survival curve slope. *z*-values can be calculated as follows:
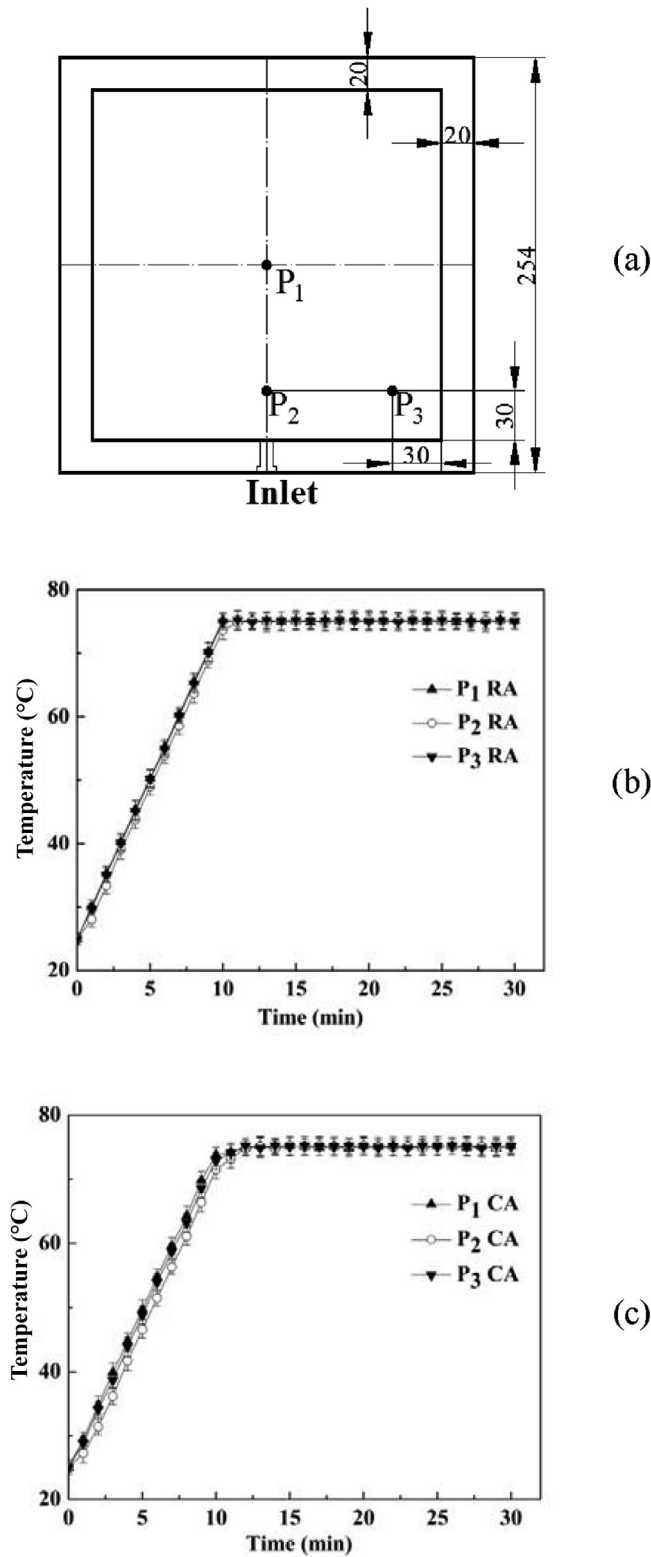
(a)

(b)

(c)

Fig. 2. Temperature comparisons of HBS (a) under RA (b) and CA (c) treatments at three positions of P_1, P_2, and P_3 with heating rate of 5 °C/min and flow rate of 1000 ml/min.

$$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}} \qquad (2)$$

Where D_{T1} and D_{T2} are decimal reduction times measured at temperatures of T_1 and T_2. z-values were determined from the reciprocal of the slope obtained by plotting the log of the D-values against exposure temperatures. The mean D- and z-values at different heating rates under RA and CA environment were separated at $P = 0.05$ level using least significant difference (LSD) test.

Both the enumeration of E. coli inoculum in almond powder and the survivors after treatments were evaluated as follows: 2 g treated almond powder was added to 48 ml sterile physiologic saline, and shaken 5 min by vortex mixture (XW-80A, Linbei Instrument Manufacturing Co., Haimen, China). Subsequently, 1 ml of E. coli ATCC 25922 bacterial suspensions were taken from conical flask and then 10-fold serial dilutions were performed in 9 ml of sterile physiologic saline; 100 μl of each one was spread onto duplicated LB agar and incubated at 37 °C for 24 h, and cultures were counted to enumerate the plates with 30−300 colonies.

2.5. Statistical analysis

Data were analyzed using the statistical product and service solutions software (V 17.0, SPSS Inc., Chicago, IL, USA). The Duncan multiple range test was used to determine significant differences at $P = 0.05$ level among means.

3. Results and discussion

3.1. Stability of the CA-HBS

3.1.1. Gas tightness of the CA-HBS
Table 1 shows the relative gas tightness of the CA-HBS based on flow meter readings. No obvious gas leakage was observed since the gas flow rate entered the heating block from the meter 4 matched the set-point values well. However, the flow rate of the gas leaving the heating block for the meter 5 was reduced and the average leakage rate was 19.10 ± 0.05 ml/min, indicated that some leakage (<2.2%) occurred, and this was probably caused by an imperfect seal between the two block surfaces. It is less than 2.4% leakage observed by Li et al. (2015a,b) and in good agreement with the results measured in the similar CA-HBS by Neven et al. (2012).

3.1.2. Temperature stability
As shown in Fig. 2b and 2c, the temperatures of P_1, P_2 and P_3 were not significantly different ($P > 0.05$) for RA treatment, while P_2 was significantly ($P < 0.05$) lower than that of P_1 and P_3 for CA treatment during the come-up stage. The maximum temperature difference in RA treatment was 2.4 °C during the come-up stage and after 2.5 min holding, temperatures of three points were stable at 75 ± 0.2 °C. While in CA treatment, the maximum temperature difference was 3.3 °C during the come-up stage and after 2.5 min holding, temperatures of three points were stable at 75 ± 0.5 °C. Table 2 shows the final populations of E. coli ATCC 25922 in almond powder when the sample temperature just reached 75 °C and after holding 2.5 min under RA and CA treatments. The final populations of E. coli at P_1, P_2 and P_3 were significantly different ($P < 0.05$) when just reached 75 °C both in RA and CA treatments, but no significant difference ($P > 0.05$) was observed after holding 2.5 min. The temperature-time history curve shown in Fig. 3 during heating, holding and cooling periods also suggested that the temperatures were stable after holding for 2.5 min. Therefore, holding for 2.5 min after come-up time could be used as time zero to provide close-to-ideal isothermal conditions.

3.1.3. Stability of O_2 and CO_2 concentrations
The variations of gas concentrations at ambient room temperature (25 °C) are shown in Fig. 4a−c. At the three gas set points, O_2

Table 1
Flow rate values (mean ± SD) taken at room temperature (25 °C) and estimated gas leakage within the CA-HBS.

Flow rate (ml/min)			Relative leakage (%)
N_2 (meter 3)	Entering HBS (meter 4)	Exiting HBS (meter 5)	
350	900 ± 0	880.65 ± 2.36	2.15
450	1000 ± 0	982.40 ± 2.84	1.98
550	1100 ± 0	1080.64 ± 3.48	1.76

concentrations variations (within ±0.056%) were more stable than those (±0.147%) of CO_2 concentrations. Mean concentrations (±standard error, SE) over 60 min were 24.996 ± 0.020%/ 0.988 ± 0.059%, 14.953 ± 0.025%/4.905 ± 0.068% and 0.976 ± 0.047%/19.721 ± 0.068% at the setpoints of 25% CO_2/1% O_2, 15% O_2/5% O_2 and 1% CO_2/20% O_2, respectively. Fig. 4d compares concentrations of the gas entering and exiting the chamber over time. The average difference in O_2 concentrations between entering and exiting the system was small (0.018%). CO_2 concentrations entering the system were higher than those exiting the system, resulting in an average difference of 0.215%. The results are consistent with those in Li et al. (2015a,b) and less than the variation of 0.5% found in Neven et al. (2012).

3.1.4. Changes in MC and A_w

Fig. 5 shows the variations of A_w and MC for RA and CA treatments. The MC of almond powder changed from initial 6.01 ± 0.08% to 5.83 ± 0.07% and 5.82 ± 0.07% for RA and CA, respectively. The A_w of almond powder varied from 0.604 ± 0.02 to 0.556 ± 0.01 and 0.554 ± 0.01 for RA and CA, respectively. The variations in test were no more than 0.18%, suggested negligible microbial population variations after heating for 2 h (Villa-Rojas et al., 2013).

3.2. Effects of O_2 concentrations on D- and z-values

D-values of E. coli ATCC 25922 under different O_2 concentrations are shown in Fig. 6. D-values decreased with increasing temperatures at every given O_2 concentration, and similar results on effects of storage temperatures were found in Abd et al. (2012). The D-values of E. coli ATCC 25922 decreased first and then increased when O_2 concentrations decreased from RA (21%) to zero at every given temperature, and reached a minimum at 2%. There were no significant (P > 0.05) differences in D-values when O_2 concentrations were ≤5% or ≥ 10%, at 65, 70 and 80 °C. But D-values with O_2 concentrations at 2% were significantly different (P < 0.05) from those with 5% and 0% at 75 °C. D_{65}-, D_{70}-, D_{75}-, and D_{80}-values at 2% O_2 concentrations decreased to 42 ± 1.5, 25.6 ± 1.4, 14.5 ± 1.3, and 8.7 ± 1.2 min, corresponding to 40.8%, 39.1%, 30.1%, and 28.3% reductions from RA (21%) conditions, respectively. The results indicated that 2% O_2 concentration might hold potential as an
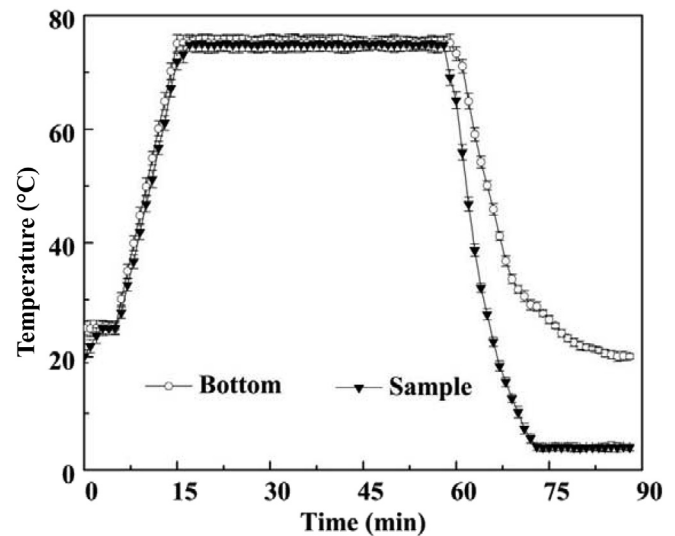

Fig. 3. Temperature-time history of almond powder samples and bottom plate during heating, holding and cooling (using 4 °C refrigerator and forced air cooling, respectively) periods.

antimicrobial treatment condition.

Table 3 shows z-values of E. coli ATCC 25922 under different O_2 concentrations. There were no significant differences (P > 0.05) when O_2 concentrations were ≤5% or ≥ 15%. z-values of E. coli ATCC 25922 at lower O_2 concentrations (≤5%) were larger than those of high O_2 concentrations (≥15%), indicated that relatively higher temperatures were needed for 1-log population reduction at lower O_2 concentrations.

3.3. Effect of CO_2 concentrations on D- and z-values under 2% O_2 condition

Fig. 7 shows D-values of E. coli ATCC 25922 under different CO_2 concentrations with 2% O_2 concentration. The higher temperatures reduced 1-log reduction time at every given CO_2 concentration, similar to the results found by Abd et al. (2012) and Yang et al. (2010). The D-values of E. coli ATCC 25922 decreased with increasing CO_2 concentrations at every given temperature. D_{65}-, D_{70}-, D_{75}-, D_{80}-values of E. coli ATCC 25922 under 20% CO_2 concentrations decreased to 35 ± 1.4, 17.8 ± 1.2, 8.6 ± 1.0, and 7.0 ± 0.8 min, corresponding to 42.5%, 40.8%, 51.4%, and 37.5% reductions from RA (21%) conditions, respectively. The results indicated that the combined CA concentration (2% O_2/20% CO_2) could be used as an effective antimicrobial treatment condition.

Table 4 shows z-values of E.coli ATCC 25922 under different CO_2 concentrations. The z-values of E.coli decreased with increasing CO_2 concentrations. The results were similar to the z-values of Listeria
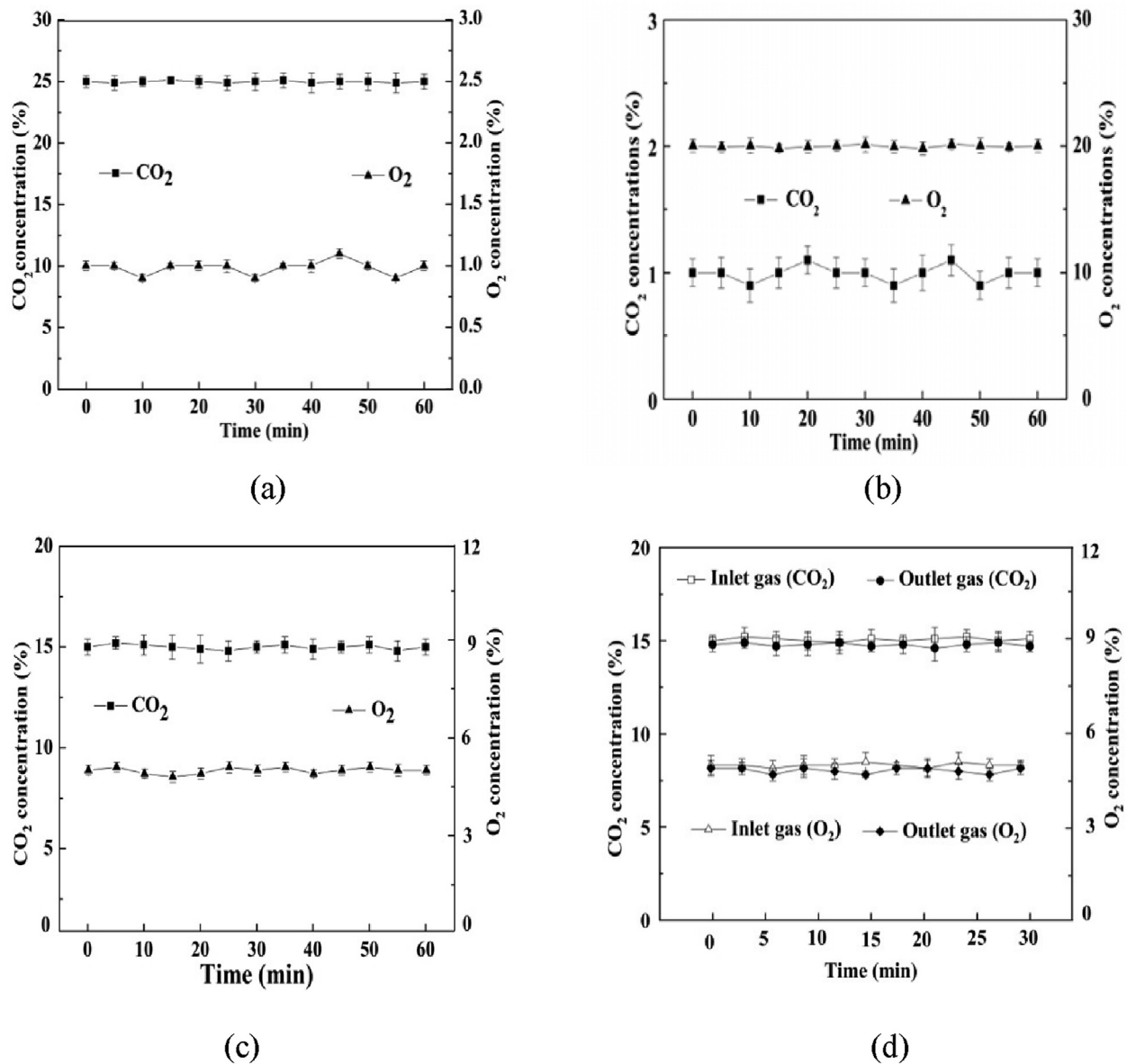
Table 2
Final populations of E. coli ATCC 25922 in almond powder with 6% w.b. located at P_1, P_2, and P_3 as shown in Fig. 2a when heated from 25 °C to 75 °C with 5 °C/min for holding 0 and 2.5 min under RA and CA treatments, respectively.

State	Temp (°C) + time (min)	Initial population (10^{10} CFU/g)	Final population (10^{10} CFU/g)		
			P_1	P_2	P_3
RA	75 + 0	3.17 ± 0.08 a[a]	2.92 ± 0.10 c	3.06 ± 0.12 a	2.95 ± 0.10 b
	75 + 2.5	3.15 ± 0.10 a	2.59 ± 0.12 b	2.55 ± 0.10 bc	2.53 ± 0.12 c
CA	75 + 0	3.16 ± 0.12 a	2.63 ± 0.10 d	2.98 ± 0.08 b	2.81 ± 0.08 c
	75 + 2.5	3.14 ± 0.08 a	2.28 ± 0.08 c	2.31 ± 0.10 bc	2.36 ± 0.10 b

[a] Different letters within a row indicate that means of final populations at initial one, P_1, P_2, and P_3 are significantly different (P < 0.05).

Fig. 4. Gas concentrations measured by O_2/CO_2 analyzer at room temperature (25 °C) and three set-point concentrations: (a) 25% CO_2 and 1% O_2, (b) 15% CO_2 and 5% O_2, (c) 1% CO_2 and 20% O_2, and (d) comparison of gas concentrations between coming into and going out the HBS and the set-point concentrations of 15% CO_2 and 5% O_2.

influenced by storage atmosphere (Juneja, 2003), indicated that higher CO_2 concentrations can reduce temperatures for achieving 1-log population reduction of *E. coli* ATCC 25922.

3.4. Effectiveness of pasteurization

The heat inactivation kinetics of *E. coli* ATCC 25922 in almond powder is shown in Fig. 8. The results indicated that the slopes increased with increasing temperatures both in RA and CA treatments, and similar trends were reported by Villa-Rojas et al. (2013). The slopes for CA treatments were larger than RA treatments at four given temperatures. The 5-log reduction time needed for CA treatments decreased to 175, 90, 43 and 35 min at 65 °C, 70 °C, 75 °C and 80 °C, respectively, by reducing 42.5%, 40.8%, 51.4% and 37.5% when compared with RA treatments. The results indicated that the *E. coli* ATCC 25922 becomes less resistant (lower *D*-values) and more sensitive (lower *z-values*) when the controlled

atmosphere (2% O_2/20% CO_2) was used.

3.5. Influence of heating rates on D-values of E. coli ATCC 25922

Fig. 9 shows the effect of five heating rates on *D*-values of *E. coli* ATCC 25922 at 75 °C under RA and CA conditions. The time from the initial temperature (25 °C) to the final temperature (75 °C) was 500, 100, 50, 10, and 5 min when the heating rates were 0.1, 0.5, 1, 5, and 10 °C/min, respectively. There were no significant differences (*P* > 0.05) in *D*-values when heating rates were ≥1 °C/min both in RA and CA treatments. In RA treatment, the *D-values* were larger with the slower heating rates, and similar trends were reported by Kou et al. (2016). The thermal resistance of *E. coli* ATCC 25922 was enhanced at lower heating rates by conventional heating. This might be caused by heat shock proteins produced in bacteria during lengthy exposures to non-lethal temperatures (Wiegand et al., 2009). But in CA treatment, *D*-values were smaller with the
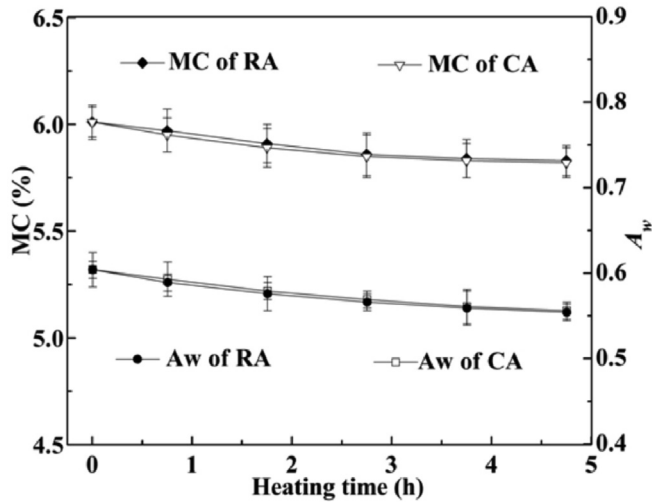
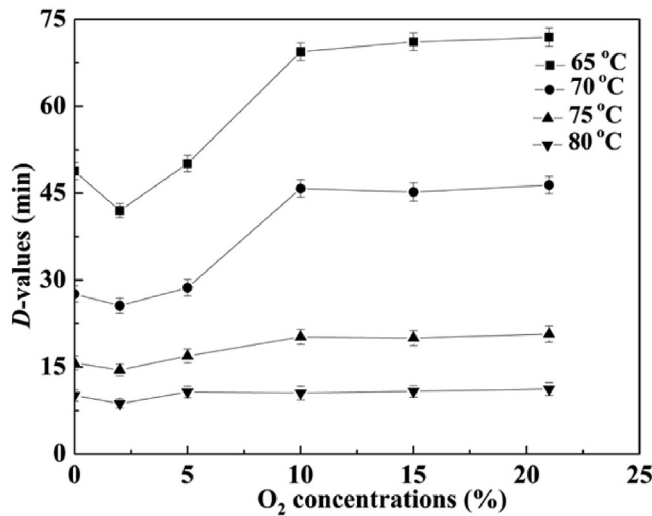Fig. 5. Changes in MC and A_w of RA and CA (2% O_2, 20% CO_2) treatments.

Fig. 6. D-values (means ± standard deviation over three replicates) of *E. coli* ATCC 25922 under different O_2 concentrations.

Fig. 7. D-values of *E. coli* ATCC 25922 under different CO_2 concentrations with 2% O_2 concentration.

Table 3
Fitting equations and *z-values* of *E. coli* ATCC 25922 under different O_2 concentrations.

O_2 (%)	Fitting equations	z-value (°C)	R^2
0	$y = -0.0227\,x + 3.280$	44.1 ± 1.1 a[a]	0.986
2	$y = -0.0228\,x + 3.293$	43.8 ± 1.3 a	0.973
5	$y = -0.0231\,x + 3.270$	43.2 ± 1.6 a	0.984
10	$y = -0.0253\,x + 3.892$	39.6 ± 1.4 b	0.975
15	$y = -0.0258\,x + 3.889$	38.7 ± 0.8 bc	0.973
21	$y = -0.0264\,x + 3.870$	37.9 ± 1.0 c	0.975

[a] Different letters within a column indicate that means of *z-values* were significantly different at $P = 0.05$ among different O_2 concentrations.
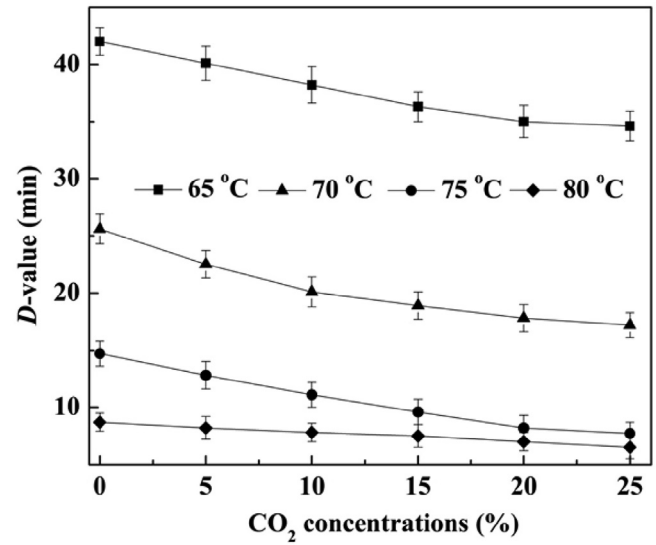
Table 4
Fitting equations and *z-values* of *E. coli* ATCC 25922 under different CO_2 concentrations with 2% O_2 concentration.

CO_2 (%)	Fitting equations	z-value (°C)	R^2
0	$y = -0.0224\,x + 3.252$	44.6 ± 1.2 a[a]	0.979
5	$y = -0.0228\,x + 3.239$	43.8 ± 1.1 ab	0.966
10	$y = -0.0231\,x + 3.172$	43.2 ± 0.9 b	0.950
15	$y = -0.0236\,x + 3.193$	42.3 ± 1.2 c	0.952
20	$y = -0.0238\,x + 3.204$	41.9 ± 1.1 cd	0.956
25	$y = -0.0239\,x + 3.197$	41.7 ± 1.0 d	0.945

[a] Different letters within a column indicate that means of *z-values* were significantly different at $P = 0.05$ among different CO_2 concentrations.
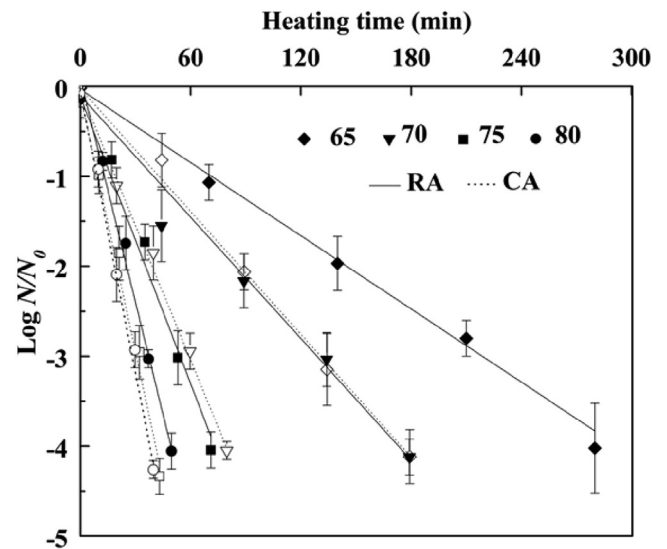

Fig. 8. Heat inactivation kinetics of *E. coli* ATCC 25922 in almond powder with 6.0% w.b. at 4 given temperatures (°C) under regular air (RA) and controlled atmosphere (CA: 2% O_2/20% CO_2).

slower heating rates. The lowest heating rate (0.1 °C/min) achieved the smallest D-values under the CA treatment, which might be caused by the lengthy exposure to CA treatment (2% O_2/20% CO_2) during the extended ramp periods.
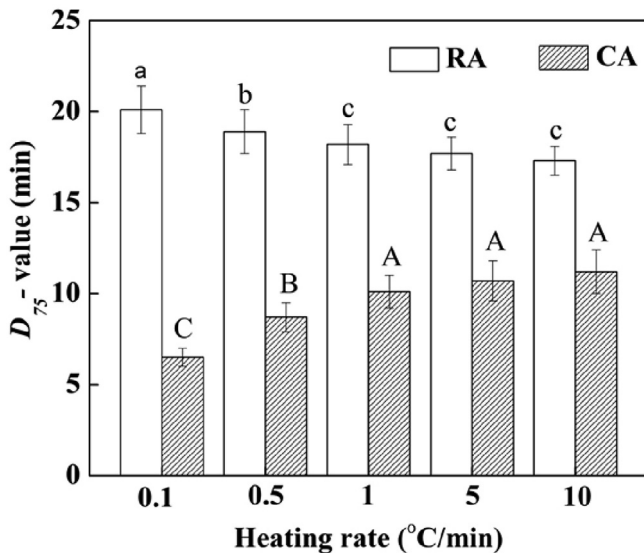
Fig. 9. *D*-values of *E. coli* ATCC 25922 at 75 °C as influenced by different heating rates under RA and CA (2% O_2/20% CO_2) treatments. Different lower and upper case letters indicate that means are significantly different at P = 0.05 among RA and CA treatments, respectively.

4. Conclusions

The stability of gas tightness, gas concentrations, temperatures and the accuracy of controlling heating rates suggest that the CA-HBS could be used in determining thermal inactivation of *E. coli* ATCC 25922 under RA and CA treatments. The *D*- and *z*-values of *E. coli* ATCC 25922 under the RA treatments were significantly ($P < 0.05$) higher than those under the CA treatments. The results demonstrated that the CA concentration of 2% O_2/20% CO_2 held at 75 °C for 43 min could reach 5-log reduction of *E. coli* ATCC 25922 for achieving pasteurization requirement. The slowest heating rate (0.1 °C/min) resulted in the highest *D*-value under the RA treatment, but lowest *D*-value under the CA treatment. Combined fast heat and CA treatments might hold potential to reduce thermal inactivation time of *S. enteritidis* PT 30 at the same target temperature.

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