Journal of Food Protection, Vol. 74, No. 7, 2011, Pages 1161–1168
doi:10.4315/0362-028X.JFP-10-415

Thermal Inactivation of Avian Influenza Virus and Newcastle Disease Virus in a Fat-Free Egg Product

REVIS A. CHMIELEWSKI, JOAN R. BECK, AND DAVID E. SWAYNE*

U.S. Department of Agriculture, Agricultural Research Service, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, Georgia 30605, USA

MS 10-415: Received 28 September 2010/Accepted 3 March 2011

ABSTRACT

High-pathogenicity avian influenza (HPAI) virus, low-pathogenicity avian influenza (LPAI) virus, virulent Newcastle disease virus (vNDV) and low-virulent Newcastle disease virus (lNDV) can be present on the eggshell surface, and HPAI viruses and vNDV can be present in the internal contents of chicken eggs laid by infected hens. With the increase in global trade, egg products could present potential biosecurity problems and affect international trade in liquid and dried egg products. Therefore, the generation of survival curves to determine decimal reduction times (D_T-values) and change in heat resistance of the viruses (z_D-value) within fat-free egg product could provide valuable information for development of risk reduction strategies. Thermal inactivation studies using A/chicken/Pennsylvania/1370/83 (H5N2) HPAI virus resulted in D_{55}-, D_{56}-, $D_{56.7}$-, D_{57}-, D_{58}-, and D_{59}-values of 18.6, 8.5, 3.6, 2.5, 0.4, and 0.4 min, respectively. The z_D-value was 4.4°C. LPAI virus A/chicken/New York/13142/94 (H7N2) had D_{55}-, $D_{56.7}$-, D_{57}-, D_{58}-, D_{59}-, and D_{60}-values of 2.9, 1.4, 0.8, 0.7, 0.7, and 0.5 min, respectively, and a z-value of 0.4°C. vNDV avian paramyxoviruses of serotype 1 (AMPV-1)/chicken/California/212676/2002 had D_{55}-, D_{56}-, $D_{56.7}$-, D_{57}-, D_{58}-, and D_{59}-values of 12.4, 9.3, 6.2, 5, 3.7, and 1.7 min, respectively. The z_D-value was 4.7°C. lNDV AMPV-1/chicken/United States/B1/1948 had D_{55}-, D_{57}-, D_{58}-, D_{59}-, D_{61}-, and D_{63}-values of 5.3, 2.2, 1.1, 0.55, 0.19, and 0.17 min, respectively, and a z_D-value of 1.0°C. Use of these data in developing egg pasteurization standards for AI and NDV-infected countries should allow safer trade in liquid egg products.

Avian health issues, principally Newcastle disease and high-pathogenicity avian influenza (HPAI), have limited global trade in poultry and poultry products. Newcastle disease is caused by avian paramyxoviruses of serotype 1 (APMV-1), of which some virulent forms of Newcastle disease virus (vNDV) infections can cause nearly 100% mortality in unvaccinated chickens, but other vNDV strains producing lower serious disease mortality rates have been used as non–tariff trade barriers. By comparison, low-virulent or lentogenic APMV-1 viruses (lNDV) can cause asymptomatic respiratory or intestinal infection, and on occasion, respiratory disease in poultry. vNDV strains have caused sporadic cases of human self-limiting conjunctivitis *(1)*.

Avian influenza (AI) is caused by infection of influenza virus A. Low-pathogenicity AI (LPAI) viruses typically cause asymptomatic infections, or at times, respiratory disease; drops in poultry egg production can be caused by any of the 16 hemagglutinin subtypes (H1 to H16). Conversely, HPAI viruses (limited to H5 and H7 subtypes) have caused severe disease in chickens and turkeys; the virus spreads to internal organs, resulting in a high fatality rates. In humans, H5, H7, and H9 AI viruses have caused sporadic infections after direct contact with infected birds, with high fatality rates for the H5N1 HPAI viruses (Guangdong lineage). vNDV and HPAI viruses result in severe economic losses to the poultry industries, disrupt trade in poultry and poultry products, and with some strains, have the potential to affect public health by causing mild to severe infections *(1, 12, 22, 31, 32)*.

HPAI viruses and vNDV pose some risk for dissemination in fresh and frozen raw poultry products, but the probability is remote. These viruses have been recovered from egg surfaces and internal egg contents, blood, and bones from infected chickens *(11, 29)*. More specifically, H5N1 HPAI viruses have been recovered from chicken thigh and breast meat, internal organs, and eggs produced by acutely infected chickens *(8, 27)*. In Korea, Japan, and Germany, H5N1 HPAI was recovered from frozen duck meat, but the contaminated products identified in Korea and Japan were imported from China *(18)*. In 1948, the importation of frozen chicken from Poland was implicated as the source of vNDV responsible for the Newcastle disease outbreak on poultry farms in England *(1)*. In contrast, LPAI viruses have not been detected in eggs or chicken meat, and the transmission of lNDV via eggs is unknown. AI virus and NDV survival in the environment and within materials is temperature and moisture dependent, wherein survival is shortened by high environmental temperatures and dry conditions *(10, 13, 16, 17, 21, 29, 33)*.

* Author for correspondence. Tel: 706-546-3433; Fax: 706-546-3161; E-mail: david.swayne@ars.usda.gov.

Egg products are traded globally for use in various food manufacturing processes. The U.S. Department of Agriculture (USDA) and Food and Drug Administration (FSIS) *(6)* mandates that commercial egg products must be pasteurized at specified time and temperature parameters to reduce pathogens below a zoonotic level. Pasteurization also extends the shelf life of commercial egg products. Although AI and AMPV-1 viruses are heat labile, the thermal inactivation of viruses is influenced by factors such as properties of the food product, the thermal stability of the strain or pathotype, and the process time–temperature. The FSIS pasteurization standards for egg products are based on a 5-log reduction of *Salmonella enterica* Enteritidis *(6)*. The pasteurization standard for plain whole egg is 60°C for 3.5 min, plain yolk is 60°C for 6.2 min, whole-egg blends is 61.1°C for 3.5 min, egg white with unadjusted pH is 57.7°C for 6.3 min, and egg white with adjusted pH is 4.3 min *(15)*. Other reformulated egg products such as imitation egg product (IEP), freeze-dried products, omelet mixes, eggnog mixes, noodles, and cake mixes are under the jurisdiction of the U.S. Food and Drug Administration (FDA), whose egg product inspection is voluntary *(4, 5, 7)*. These products are usually made from pasteurized egg products, and the law does not require repasteurization after reformulation. Despite this, manufacturers of egg products for direct consumption usually perform a second pasteurization process. In the United States, liquid egg white products constitute 24% of further-processed egg products *(22)*. Reformulated egg products such as IEP consist of at least 98% egg white and can contain up to 2% added ingredients *(3)*. The high percentage of egg white in IEP limits the temperature of pasteurization in order to maintain a desirable consistency and viscosity. The pasteurization recommended for IEP is 56.7°C for 4.6 min *(15)*.

The increased incidence of H5N1 HPAI poultry infections from some Asian, Eastern European, Middle Eastern, and African countries, along with the increase in global trade, raises concern that some poultry products could potentially pose biosecurity risks and affect (or have affected) trade. There is a need to develop standards for egg product pasteurization to inactivate AI viruses and NDV in various chicken egg products. The objective of this study was to develop predictive models for the inactivation of both AI viruses and NDV within a fat-free liquid egg product at various time and temperature heat processes. Linear and nonlinear regression models were used to determine thermal death times (D-values) and thermal resistance (z_D-values) of the viruses in fat-free egg product (FFEP). In addition, the experimental inactivation data of both AI viruses and NDV within a fat-free liquid egg product were validated against the FSIS pasteurization standard for *Salmonella* in IEP (56.7°C for 4.6 min), and the thermal process (F value) was calculated. The F value was also calculated with our predictive data at 57.7°C, along with the pasteurization standard for egg white without pH adjustment (57.7°C for 6.3 min) *(15)*. This study was based on one pasteurization cycle.

MATERIALS AND METHODS

Virus inoculum preparation. HPAI virus A/chicken/Pennsylvania/1370/83 (H5N2) (HPAI/PA/83), LPAI virus A/chicken/New York/13142-5/94 (H7N2) (LPAI/NY/94), vNDV APMV-1/chicken/California/212676/2002 (vNDV/CA/02), and lNDV APMV-1/chicken/United States/B1/1948 (lNDV/B1/48) were propagated by inoculation of 10-day-old, specific pathogen–free, embryonating white leghorn chicken eggs (Southeast Poultry Research Laboratory, Athens, GA) in chorioallantoic sacs. Titration of the virus stock was completed with standard tissue culture methods *(28)* and expressed as 50% tissue culture infectious doses (TID_{50}) *(25)*.

Sample preparation. Samples of FFEP (AllWhites Egg Whites, Crystal Farms, Lake Mills, WI) were artificially inoculated with a 1:10 dilution of virus, resulting in 10^6 to $10^{8.5}$ TID_{50}. Ninety-microliter aliquots of the artificially infected homogenized egg product were transferred into 200-μl, thin-walled thermocycler polypropylene tubes (Thermowell, Corning, Inc., Lowell, MA) and maintained at 4°C.

Heat inactivation. Samples were equilibrated at 25°C for 2 min and then heated to the desired temperature. On reaching the desired temperature, samples at time zero were removed, and the timing for pasteurization was commenced. The average come-up time to test temperature was 30 s. Samples were heated at 53, 55, 57, 58, 59, 61, and/or 63°C for 0, 1, 2, 3, 4, 6, 8, 12, and sometimes at 15 to 40 min in a PCR thermocycler heating block (GeneAmp 9700, PerkinElmer, Boston, MA), and were then cooled immediately to 4°C in a cooling block. For some sampling times, triplicate samples were pooled to obtain a final volume of 270 μl. The purpose of the pooling was to obtain sufficient volume to titer the undiluted sample (10^0) and to decrease the detection limit to 1 log. Samples were serially diluted (10^{-1} to 10^{-8}) in GIBCO Dulbecco's modified essential medium (Invitrogen, Carlsbad, CA) with 1× antibiotic-antimycotic (HyClone, Thermo Scientific, Suwannee, GA) for inoculation into cell culture for determining infectious titer. Each experiment was replicated three times.

Tissue culture assay. Monolayers of chicken embryo fibroblast cells were used to titer AMPV-1 (NDV), and Madin-Darby canine kidney cells were used to titer AI viruses in 96-well microtiter plates. Monolayers were washed three times with phosphate-buffered saline, inoculated with 50 μl of virus inoculum, and incubated for 1 h. After incubation, 100 μl of medium supplemented with GIBCO trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA; Invitrogen) was added to the inoculated monolayer and incubated at 37°C, with 5% CO_2, humidified atmosphere for 4 days. Titers of viruses were determined with a hemagglutination assay. In short, a 100-μl aliquot of supernatant from the inoculated tissue culture was transferred into a microtiter plate, 50 μl was serially diluted (1:2), and then overlaid with 0.5% chicken red blood cells. After 30 min of incubation, the presence or absence of agglutination was recorded. Virus titer was determined by calculation of the TID_{50} *(25)*.

Statistical data analysis. Inactivation curves were generated by plotting the log value of the TID_{50} as a function of the heating time at that temperature by using Excel 2003 (Microsoft Corp., Redmond, WA). The inactivation curves were examined to determine the best-fit statistical distribution. Linear, exponential, and polynomial curves were obtained. The D-values, or times (expressed in minutes) to inactivate the viral load by 90%, were calculated from the inverse value of the slope (1/–slope) of the linear regression plot. The D-values for the exponential plot were

TABLE 1. *Experimental* D_T*-values in FFEP for HPAI/PA/83*[a,b]

Temp (°C)	D_T-value (min)	SD[c]	Equation	R^{2d}
55	18.6	±0.01	$y = -0.0037x^2 + 0.0465x + 5.897$	0.96
56	8.5	±0.03	$y = -0.0149x^2 - 0.0876x + 5.884$	0.98
56.7	3.6	±0.02	$y = -0.0137x^2 - 0.251x + 6.0209$	0.99
57	2.5	±0.02	$y = -0.0124x^{2-} - 0.374x + 6.1291$	0.98
57.7	1.1[e]	NA[f]	NA	NA
58	0.4	±0.16	$y = -0.2286x^2 - 0.2057x + 5.6429$	0.91
59	0.4	±0.43	$y = -1.268x^2 - 0.0149x + 5.2577$	0.99

[a] D_T-values are the times required to reduce virus titer by 90% or 1 log TID_{50}.
[b] Experiments were replicated three times. The z_D-value was 4.3 ± 0.03°C, with its equation being $y = -0.3565x + 1.6407$, and its R^2 being 0.95.
[c] Standard deviations (SD) were generated from the thermal inactivation equation of HPAI-A/ck/PA/1370/83 (H5N2) infected with an initial titer of 6.25 log (TID_{50}/ml) in FFEP.
[d] The correlation coefficient (R^2) indicated the goodness of fit of the predictive equation.
[e] D_T predicted from plot of log D_T-values versus temperature.
[f] NA, not applicable.

based on inverse value of the exponential of the (slope); for polynomial distribution, the slopes were calculated by multiplying the exponent value by the coefficient and using the inverse value. The z_D-value is the change in temperature required to change the D_T-value by 1 log or 10-fold, and was determined with Excel 2003, by computing the regression of the mean log D-values versus heating temperatures. The z_D-value is the absolute value of the inverse slope.

The F value corresponds to the time–temperature combinations necessary to reduce the concentration of the target organism to an acceptable level; this value was expressed as a given number of D-values. For example, to obtain 5-log reduction of *Salmonella* in IEP required 4.6 min at 56.7°C; hence, to achieve a 1-log reduction at the same temperature would require 4.6/5, or 0.92 min. Therefore, if the D_T-value of the experimental data (56.7°C) was 2.3 min, then the F value for that process for the experiment would be 2.3 min. If the experimental organism was treated by the standard IEP process (4.6 min at 56.7°C), the resulting log reduction would be only 4.6/2.3, or 2 log.

RESULTS

Thermal inactivation of AI viruses and NDV. The thermal inactivation study generated survival curves, from which D-values for HPAI/PA/83, LPAI/NY/94, vNDV/CA/2002, and lNDV/B1/48 were calculated (Fig. 1). The thermal inactivation data for HPAI/PA/83 at 55 and 56°C had exponential declines in viral titers (Table 1). HPAI/PA/83 was resistant to heating at 55°C, requiring at least 93 min to inactivate viral population by 5 log, but by 56°C, the 5-log reduction required 43 min of heat exposure. Our inactivation data demonstrated that heating HPAI/PA/83 at 56.7°C required at least 18 min to inactivate 5 log of virus, whereas obtaining a similar reduction at 57 and 58°C would require 13 and 2 min, respectively. Using the FSIS pasteurization standard for IEP (56.7°C, with a minimum holding time of 4.6 min to obtain a 5-log reduction of *Salmonella*), an F value could be calculated. The pasteurization process of HPAI/PA/83 in FFEP would obtain a reduction of 1.3 log or a D_T-value of 1.3 when using the IEP standard (Table 2). Meanwhile, using the pasteurization standard for egg white with unadjusted pH (57.7°C with a minimum holding time of 6.3 min) would result in a D_T-value of 5.7 (Table 2).

The results of the D-values for LPAI/NY/94 (H7N2) are presented in Table 3. At 55°C, the inactivation rate was log linear; however, the inactivation curve plateau between 3 and 6 min suggests some thermal resistance at those times. Thermal inactivation of LPAI/NY/94 at 56.7°C required at least 5 min to inactivate 5 log of virus, whereas heating at 57°C required approximately 4 min for a similar log reduction. Viral inactivation between 58 and 59°C was complete in less than 2 min. Using the IEP pasteurization process for LPAI/NY/94 in FFEP would result in a D_T-value of 4.5, while the egg white pasteurization process would result in a D_T-value of 7.8 (Table 2).

The D-values for vNDV/CA/2002 are presented in Table 4. The thermal inactivation curves generated between 55 and 59°C demonstrated a constant log-linear rate of inactivation, indicating a similar degree of resistance in the viral population. Heating at 56.7°C required at least 31 min to inactivate 5 log of virus, while total inactivation (<7 log) occurred after 15 min of exposure at 59°C in FFEP. The pasteurization process of vNDV/CA/02 in FFEP resulted in a D_T-value of <1 by using the IEP standard (Table 2); when using the egg white pasteurization standard, the pasteurization process of vNDV/CA/02 in FFEP resulted in a D_T-value of 1.7 (Table 2).

The D-values for lNDV/B1/48 are presented in Table 5. lNDV/B1/48 demonstrated some thermal resistance between 55 and 58°C. Heating at 55°C required at least 26.5 min to inactivate 5 log of virus, while at 57°C, 11 min of heating was required to inactivate 5 log. At 59°C, there was total inactivation (>6-log reduction) within 4 min. The pasteurization (56.7°C, with holding time of 4.6 min) of lNDV/B1/48 in FFEP resulted in a D_T-value of 1.4, whereas using the pasteurization standards for egg white process resulted in a D_T-value of 3.3 (Table 2).

The estimated times (in minutes) to obtain a thermal process with a D_T-value of 5 (or a 5-log reduction) in FFEP at 56.7°C were 8 min for HPAI/PA/83, 5 min for LPAI/NY/94, 31 min for vNDV, and 19 min for lNDV/B1/48

TABLE 2. D-*value averages of HPAI/PA/83, LPAI/NY/94, vNDV/CA/2002, and lNDV/B1 in FFEP obtained from the prediction models, and the predicted number of log reductions achieved at standard pasteurization parameters*[a]

Virus	Temp (°C)	D_T-value[b]			Predicted no. of virus log reductions for virus in IEP or liquid egg white[c,d]
		1 min	2 min	3 min	
HPAI/PA/83	56.7	3.6	10.8	18	1.3
LPAI/NY/94	56.7	1.0	3.0	5	4.6
vNDV/CA/2002	56.7	6.2	18.6	31	<1
lNDV/B1	56.7	3.25	9.75	16.25	1.4
HPAI/PA/83	57.7	1.1	3.3	5.5	5.7
LPAI/NY/94	57.7	0.8	2.4	4.0	7.8
vNDV/CA/2002	57.7	3.7	11.1	18.5	1.7
lNDV/B1	57.7	1.9	5.7	9.5	3.3

[a] Parameters were 56.7°C for 4.6 min for IEP or 57.7°C for 6.3 min for egg whites, with unadjusted pH.
[b] D_T-value, time required to inactivate 1 log of virus at 56.7 or 57.7°C.
[c] Predicted number of viral log reductions (F value) achieved based on the USDA pasteurization standard of 56.7°C for 4.6 min in IEP.
[d] Predicted number of viral log reductions (F value) achieved based on the USDA pasteurization standard of 57.7°C for 6.3 min in egg white, without pH adjustment.

(Table 2). Those estimated times to obtain a thermal process with a D_T-value of 5 were higher than the 4.6 min required to inactivate a 5-log population of *Salmonella*. Meanwhile, the times to obtain a process with a D_T-value of 5 in FFEP at 57.7°C would be 5.5 min for HPAI/PA/83, 4 min for LPAI/NY/94, 18.5 min for vNDV, and 9.5 min for lNDV/B1/48 (Table 2). The proposed pasteurization standard for egg white (6.3 min at 57.7°C) should be considerably more effective in inactivating the viruses in FFEP than should the IEP pasteurization requirements (56.7°C). Nevertheless, the egg white pasteurization standard should be inadequate in inactivating a 5-log population for lNDV and vNDV, yet be effective in inactivating the 5-log population of both LPAI and HPAI within the required 6.3 min.

Modeling of thermal kinetics and determining the changes in thermal resistance. Our data for the inactivation of AI viruses and NDV were modeled with first-order kinetics or linear and nonlinear regression. Log-linear inactivation suggests a similar rate of inactivation within the viral population, but when the inactivation curves are up or down, as in an exponential or polynomial decline, the assumption is that there are variable degrees of resistance within the population. The change in thermal resistance of the virus for a temperature range (z_D-value) was calculated based on the plotting of the log D-values of the virus versus heating temperatures (in degrees Celsius) and using the absolute value of the inverse slope. Again, the z_D-value is defined as the change in temperature (in degrees Celsius) required to change the viral population by 1 log.

The z_D-values for HPAI/PA/83, LPAI/NY/94, vNDV/CA/2002, and lNDV/B1/48 are presented in Tables 1, 3, 4, and 5, respectively. The thermal death time curves for HPAI/PA/83 virus revealed an exponential rate of decline. The inactivation curve had a downward concave, suggesting that the remaining viral population became increasingly more susceptible to heat. However, the cumulative degree of thermal resistance between 55 and 59°C resulted in a z_D-value of 4.3°C, which indicates significant resistance of HPAI/PA/83 to differing temperatures (Table 1 and Fig. 1A). Comparatively, the z_D-value for LPAI/NY/94 with a change <0.5°C resulted in a 1-log decrease in the

TABLE 3. *Experimental* D_T*-values in FFEP for LPAI/NY/94*[a,b]

Temp (°C)	D_T-value (min)	SD[c]	Equation	R^{2d}
55	2.9	±0.03	$y = -0.348x + 5.435$	0.96
56.7	1.0	±0.06	$y = 0.13x^2 - 1.235x + 3.873$	0.99
57	0.8	±0.11	$y = 0.255\ x^2 - 1.763x + 3.872$	0.95
57.7	0.72[e]	NA[f]	NA	NA
58	0.6	±0.14	$y = 1.29x^2 - 3.489x + 3.4455$	0.99
59	0.5	±0.25	$y = -2x + 2.83$	0.92
61	0.4	±0.33	$y = -2.46x + 2.23$	0.99

[a] D_T-values are the times required to reduce virus titer by 90% or 1-log TID_{50}.
[b] Experiments were replicated three times. The z_D-value was 0.4 ± 0.03°C, with its equation being $y = 0.0275x^2 - 3.33x + 100.45$, and its R^2 being 0.99.
[c] Standard deviations (SD) were generated from the thermal inactivation equation of LPAI-A/ck/NY/13142/94 (H7N2) infected with an initial viral titer of 5.6 log (TID_{50}/ml) in FFEP.
[d] The correlation coefficient (R^2) indicated the goodness of fit of the predictive equation.
[e] D_T predicted from plot of log D_T-values versus temperature.
[f] NA, not applicable.

TABLE 4. *Experimental* D_T*-values in FFEP for vNDV/CA/2002*[a,b]

Temp (°C)	D_T-value (min)	SD[c]	Equation	R^{2d}
55	12.4	±0.005	$y = -0.081x + 8.630$	0.97
56	9.3	±0.007	$y = -0.1077x + 8.4224$	0.96
56.7	6.2	±0.02	$y = -0.162x + 8.431$	0.98
57	5.0	±0.05	$y = -0.198x + 8.392$	0.99
57.7	3.68[e]	NA[f]	NA	NA
58	3.7	±0.01	$y = -0.281x + 8.135$	0.98
59	1.7	±0.03	$y = -0.603x + 8.406$	0.98

[a] D_T-values are the times required to reduce virus titer by 90% or 1-log TID_{50}.
[b] Experiments were replicated three times. The z_D-value was 4.7 ± 0.02°C, with its equation being $y = -0.213x + 12.858$, and its R^2 being 0.97.
[c] Standard deviations (SD) were generated from the thermal inactivation equation for vNDV chicken/U.S.(CA)/212676/2002 artificially infected with an initial titer of 8.75 log (TID_{50}/ml) in FFEP.
[d] The correlation coefficient (R^2) indicated the goodness of fit of the predictive equation.
[e] D_T predicted from plot of log D_T-values versus temperature.
[f] NA, not applicable.

viral population (Table 3 and Fig. 1B), indicating that the LPAI virus is more sensitive to the changes in temperature than HPAI/PA/83. Similarly, for NDV, the highly virulent vNDV/CA/2002 was significantly more resistant to temperature changes than the lentogenic lNDV/B1/48 virus (Tables 4 and 5, and Fig. 1C and 1D).

DISCUSSION

The United States produces more than 200 million cases of shell eggs per year, of which 30.8% of them are processed as liquid egg product including liquid whole egg, liquid egg white, fortified whole eggs and yolk, sugared and salted whole eggs and yolks, and various blends of egg products *(3)*. The majority of liquid egg products is for commercial purposes and must be pasteurized before distribution for consumption, but not all egg products are required by law to be pasteurized *(30)*. Those exempt from the law include freeze-dried eggs, imitation eggs, and egg substitutes. These products are usually made from pasteurized eggs, but manufacturers are not mandated to repasteurize the formulated products. These egg products are under FDA jurisdiction *(30)*. IEP or egg substitute consists of mostly egg white (>98%), with some products being fat-free, while others might contain up to 2% added ingredients such as unsaturated fats and oils, vitamins, gums (xanthan, guar), and nonfat dry milk *(3)*. According to the USDA National Nutrient Database for Standard Reference, liquid or frozen fat-free egg substitute nutrient value consists of water, protein, total fat, carbohydrate, calcium, potassium, iron, sodium, vitamin A, and vitamin C (as reported in Table 6). These are similar to the content of the FFEP used in this study. By contrast, FFEP is >98% egg white and thus similar to liquid egg white product.

The FDA Federal Register Final Rule of 9 July 2009 *(7)* (''Prevention of *Salmonella enterica* Enteritidis in Shell Eggs during Production, Storage, and Transport'') and the Egg Product Inspection Act referenced the use of pasteurization of egg products in accordance with USDA requirements Title 9, Code of Federal Regulations (CFR) §590.570 *(6)*. The USDA Egg Product Inspection Act (Title 21, CFR §§118.3 and 118.6) *(5)* pasteurization guideline for IEP is 56.7°C, with a holding time of 4.6 min to obtain a 5-

TABLE 5. *Experimental* D_T*-values in FFEP for INDV/B1/48*[a,b]

Temp (°C)	D_T-value (min)	SD[c]	Equation	R^{2d}
55	5.3	±0.02	$y = -0.1892x + 7.9566$	0.94
56.7	3.25	NA[e]	NA	
57	2.2	±0.07	$y = -0.0011x^4 + 0.0402x^3 - 0.4264x^2 + 0.8579x + 7.1828$	0.98
57.7	1.89[f]	NA	NA	NA
58	1.1	±0.13	$y = 0.0393x^3 - 0.4223x^2 + 0.04x + 7.5839$	0.99
59	0.55	±0.22	$y = 0.3083x^3 - 1.8089x^2 + 0.8649x + 6.7121$	0.99
61	0.19	±0.01	$y = -5.4x + 6.4$	1.0
63	0.17	±0.01	$y = -2.8x + 5.666$	0.75

[a] D_T-values are the times required to reduce virus titer by 90% or 1-log TID_{50}.
[b] Experiments were replicated three times. The z_D-value was 1.0 ± 0.15°C, with its equation being $y = -0.0047x^2 + 0.3038x - 1.6037$, and its R^2 being 0.99.
[c] Standard deviations (SD) were generated from the thermal inactivation equation for lNDV/B1/48 artificially infected with an initial titer of 6.75 log (TID_{50}/ml) in FFEP.
[d] The correlation coefficient (R^2) indicated the goodness of fit of the predictive equation.
[e] NA, not applicable.
[f] D_T predicted from plot of log D_T-values versus temperature.

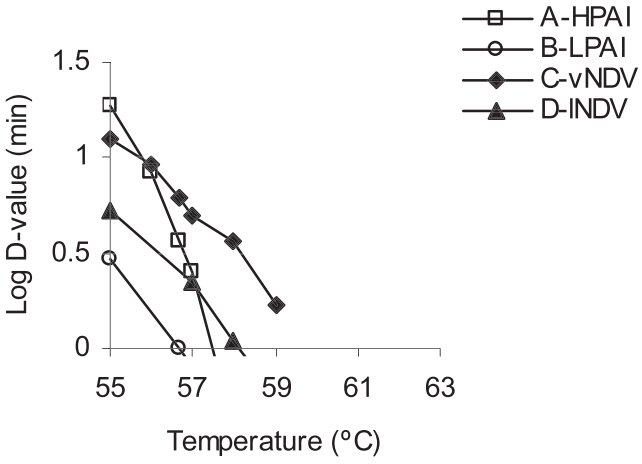

FIGURE 1. *Thermal death time curves for (A) HPAI-A/ck/PA/1370/83 (H7N2), (B) LPAI-A/ck/NY/13142/94 (H5N2), (C) vNDV chicken/U.S.(CA)/212676/2002), and (D) lNDV/B1 (chicken/U.S./B1/1948) in FFEP.*

log reduction (D_T-value of 5) of *Salmonella (3, 4).* In our study, we developed heat inactivation models for both LPAI and HPAI viruses, and lNDV and vNDV. The decimal reduction time (D_T) and thermal resistance due to changes in temperature (z_D) were obtained from the thermal inactivation models. The D_T-values at 56.7°C with holding time of 4.6 min in FFEP for both AI viruses and NDV were compared with the D_T-values of the USDA pasteurization for IEP to obtain a thermal process (F value) for FFEP. The thermal process was also calculated for FFEP, based on international egg pasteurization guidelines proposed by the United Egg Association and the American Egg Board for liquid egg white with unadjusted pH (57.7°C for 6.3 min) *(15).* The data produced were derived from one pasteurization cycle, although further-processed or reformulated egg product might be subjected to a second pasteurization process.

The thermal inactivation process in FFEP at 56.7°C, with a minimum holding time of 4.6 min showed that this pasteurization process did not achieve the 5-log population reduction as required in the pasteurization of egg products to eliminate *Salmonella*. If we used the liquid egg white standard, of which our FFEP is >98% liquid egg white, for *Salmonella* inactivation (unadjusted pH at 57.7°C for 6.3 min to inactivate 5 log) *(15),* the scenarios improved, with a better predictive model for virus inactivation. However, the quantity of virus titer reduction needed will be different for each of the four viruses. In a recent study, chicken hens infected with H5N2 HPAI virus produced eggs containing as high as 4.9 log 50% egg infectious dose (EID_{50})/ml virus on the surface of the shell, 1.2 to 4.8 log EID_{50}/ml in the yolk, and 1.5 to 6.1 log EID_{50}/ml in the egg albumen *(14).* Therefore, if we use this worst-case scenario and require IEP pasteurization standards of 6.1-log reductions for HPAI viruses, then the pasteurization temperature should be either higher than 56.7°C (the time parameter needs to be extended with longer holding time than 4.6 min) or a second pasteurization process is needed.

TABLE 6. *Nutritional content of FFEP in brand name egg product compared with the USDA national reference standard*

Nutrient	AllWhites Egg Whites (per 100 g)	USDA national database standard reference (per 100 g)
Protein	10 g	10 g
Total fat	0	0 g
Carbohydrate	1.8 g	2.0 g
Calcium	0	73 mg
Potassium	170 mg	213 mg
Iron	0	1.98 mg
Sodium	160 mg	199 mg

One limitation to this pasteurization process is that heating liquid egg white above 57°C can start the precipitation of the egg protein, resulting in coagulation at 73°C *(19)*; therefore, minimizing the holding time while increasing the processing temperature might be critical. With LPAI, virus has only been identified on the eggshell surface at 1.2 log EID_{50}/ml and not in the internal contents *(14).* This suggests that the IEP process (56.7°C, 4.6 min) would be more than adequate to inactivate completely any virus that could contaminate the albumen from the eggshell surface during the egg-cracking procedure. By contrast, there is a lack of data on the quantity of virus present on the eggshell surface and internal contents of eggs from vNDV- and lNDV-infected hens. However, because of similarities in pathogenesis of HPAI and LPAI viruses with vNDV and lNDV, respectively, the maximum vNDV quantity in albumen should be similar to the quantity for HPAI virus in egg albumen, while the lack of LPAI virus in internal contents of eggs should be expected for lNDV as well.

There are limited data available on the heat inactivation of AI viruses or NDV; therefore, some of the comparative data would be associated with *Salmonella,* the organism on which the pasteurization standard was based. Swayne and Beck *(27)* found that thermal inactivation of HPAI/PA/83 in fresh liquid egg white resulted in a $D_{56.7}$ of 33.1 s versus the 3.6 min observed with FFEP, and for vNDV/CA/02, the D_{57} was 21 s versus the 2.4 min in FFEP *(27).* The study by Swayne and Beck *(27)* demonstrated that a process resulting in a D_T-value of 5 would be achieved, thereby providing an adequate margin of safety, whereas our data of FFEP pasteurization were contrary to the fresh egg white data *(27).* The differences in D_T-values between the fresh liquid egg white study and our FFEP study could be attributed to the fact that filtering and heat processing might have reduced the functionality of inhibitory protein and enzymatic activity in FFEP, allowing the viruses an environment more suitable for survival. Fresh egg white also has higher protein content (12 to 15%) versus the FFEP protein content of 9 to 10%. The pH of both products was essentially the same; FFEP had a pH of 7.6, while fresh egg white's pH was 7.3. Similar variability in thermal inactivation data was observed in studies by Jin et al. *(20)* on the inactivation of *Salmonella* in liquid egg whites, wherein a $D_{56.6}$ of 0.37 min was recorded; Palumbo et al. *(24)* reported a $D_{56.6}$ of 1.44 min for a six-strain inoculum of *Salmonella* in egg

whites at pH 8.6. Meanwhile, Shuman and Sheldon *(26)* obtained a $D_{56.6}$ of 2.96 min for *Salmonella* in liquid egg white at pH 8.2, and a $D_{56.7}$ of 1.58 min for *Salmonella* at pH 9.1. Clearly, the thermal treatment of *Salmonella* in liquid egg white at various pH values did not achieve the thermal process with a D_T-value of 5, similar to the observation in our study. Many factors can influence the thermal stability of viruses, including strain types, heating medium, pH, microbial concentration, and experimental method *(20)*.

Previous research *(1, 27)* demonstrated that thermal stability or resistance of NDV and AI viruses might be dependent on the heating medium, for example, the change in thermal resistance value (z_D-value) for vNDV/CA/02 in whole, homogenized egg was 5.0°C, and for liquid egg whites was 4.4°C *(27)*. For HPAI/PA/03 in whole and liquid egg, the z_D-values were 3.6 and 1.9°C, respectively. In our study, the thermal resistance of the AI viruses and NDV in FFEP suggests that the HPAI and vNDV were more thermally resistant than were the low-virulent types, as a greater change in temperature was required to inactivate the viruses by 1 log. LPAI/NY/94 was the most sensitive to changes in temperature; lNDV/B1/48 was second most sensitive, whereas HPAI/PA/83 and vNDV/CA/2002 were the most resistant to changes in temperature.

In this study, the thermal inactivation models for AI viruses and NDV provide useful data, critical for quantitative risk assessments in determining the probability of viral exposure in egg products and the value of mitigation steps in consumer assurance. In previous research, HPAI/PA/83 (H5N2) was detected in egg and yolk samples in 57% of eggs laid by infected chickens during an outbreak in the northeastern United States. Unpublished work showed that eggs from HPAI virus–infected hens had an average titer of 2.6 log EID_{50}/ml, with titers as high as 6.1 log/ml for individual HPAI infected eggs, but no virus had been detected in internal contents of eggs from chickens infected with LPAI viruses. This emphasizes the importance to mitigate risk of viral transmission by setting thermal processing parameters that provide a margin of safety *(9)*. Our study showed that the virulent viruses tested were slightly more heat resistant than were the low-virulent pathotypes in FFEP, and that the thermal inactivation process at 56.7°C for 4.6 min might not adequately provide a margin of safety during pasteurization if the egg product is contaminated with >1 log of HPAI viruses or NDV. However, pasteurization at 57.7°C for 6.3 min might provide a moderate margin of safety for both HPAI and LPAI viruses, especially when considering that the come-up time to the temperature of interest inactivates at least 1 log of the virus population. Because most processed liquid egg products are pasteurized twice before reaching consumers, the possibility of egg products contamination with either AI viruses or NDV is miniscule. Moreover, there is no evidence of the spread of either lNDV or LPAI viruses by way of eggs, and the transmission of vNDV or HPAI viruses in eggs is rare. However, if such incidence were to occur, the viral titer would be considerably less than 5 log, due to the dilution effect from bulk production and the multiple sources of raw egg products. A scientific assumption more valid is to use the average titer, such as 2.6 log, for NDV- and AI virus-infected eggs rather than the maximum titer (6.1 log), because the cracked eggs are blended before the pasteurization process.

ACKNOWLEDGMENTS

The authors thank the American Egg Board for financial support and Hershell Ball of Michaels Foods for supplying products for this study.

REFERENCES

1. Alexander, D. J. 2001. Newcastle disease. *Br. Poult. Sci.* 42:5–22.
2. Alexander, D. J., and R. J. Manvel. 2004. Heat inactivation of Newcastle disease virus (strain Herts 33/56) in artificially infected chicken meat homogenate. *Avian Pathol.* 33:222–225.
3. American Egg Board. 2010. Shell egg distribution and consumption. Available at: www.aeb.org/egg-industry-facts/shell-egg-distribution-and-consumption. Accessed 15 August 2010.
4. Anonymous. 1993. Requirements for the specific standardized eggs and egg products. Title 21, Code of Federal Regulations, §2. Available at: http://www.access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200521. Accessed 19 July 2010.
5. Anonymous. 2003. Egg product inspection act: prevention of *Salmonella Enteritidis* in shell eggs during production, storage and transportation. Title 21, Code of Federal Regulations, §16 and 118. Available at: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR. Accessed 19 July 2010.
6. Anonymous. 2008. Pasteurization of liquid eggs. Title 9, Code of Federal Regulations, §590.570. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_08/9cfr590_08.html. Accessed 4 September 2009.
7. Anonymous. 2009. Shell eggs. Title 21, Code of Federal Regulations, §115. Available at: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR. Accessed 2 August 2010.
8. Beato, M. S., I. Capua, and D. J. Alexander. 2009. Avian influenza viruses in poultry products: a review. *Avian Pathol.* 38:193–200.
9. Cappucci, D. T., D. C. Johnson, M. Brugh, T. M. Smith, C. F. Jackson, J. E. Pearson, and D. A. Senne. 1985. Isolation of avian influenza virus (subtype H5N2) from chicken eggs during a natural outbreak. *Avian Dis.* 29:1195–2000.
10. Center for Food Security and Public Health. 2008. Newcastle disease: avian paramyxovirus-1 infection, goose paramyxovirus infection. Available at: http://www.cfsph.iastate.edu/Factsheets/pdfs/newcastle_disease.pdf. Accessed 30 March 2010.
11. Chen, J.-P., and C.-H. Wang. 2002. Clinical epidemiologic and experimental evidence for the transmission of Newcastle disease virus through eggs. *Avian Dis.* 46:461–465.
12. Cox, N., and T. M. Uyeki. 2008. Public health implications of avian influenza viruses, p. 453–484. *In* D. E. Swayne (ed.), Avian influenza. Blackwell Publishing, Ames, IA.
13. Domanska-Blicharz, K., Z. Minta, K. Smietanka, S. March, and T. van den Berg. 2010. H5N1 High-pathogenicity avian influenza virus survival in different types of water. *Avian Dis.* 54:734–737.
14. Eggert, D., and D. E. Swayne. 2011. Unpublished data.
15. Froning, G. W., D. Peters, P. Muriana, K. Eskridge, D. Travnicek, and S. Sumner. 2001. International egg pasteurization manual. United Egg Association and the American Egg Board, Parkview, IL.
16. Guan, Y., J. S. M. M. Peiris, K. F. Kong, K. C. Dyrting, T. M. Ellis, T. Sit, L. J. Zhang, and K. F. Shortridge. 2002. H5N1 influenza viruses isolated from geese in southeastern China: evidence for genetic re-assortment and interspecies transmission to ducks. *Virology* 292:16–23.
17. Guan, Y., J. S. M. Peiris, A. S. Lipatov, T. M. Ellis, K. C. Dyrting, S. Krauss, L. J. Zhang, R. G. Webster, and K. F. Shortridge. 2002. Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR. *Proc. Natl. Acad. Sci. USA* 99:8950–8955.
18. Harder, T. C., J. Teuffert, E. Starick, J. Gethmann, C. Grund, S. Fereidouni, M. Durban, K.-H. Bogner, A. Neubauer-Juric, R. Repper, A. Hlinak, A. Engelhardt, A. Nöckler, K. Smietanka, Z. Minta, M.

Kramer, A. Globig, T. C. Mettenleiter, F. J. Conraths, and M. Beer. 2009. Highly pathogenic avian influenza virus (H5N1) in frozen duck carcasses, Germany, 2007. *Emerg. Infect. Dis.* 15:1–8.
19. Herald, T. J., F. A. Osorio, and D. M. Smith. 1989. Rheological properties of pasteurized liquid whole egg during frozen storage. *J. Food Sci.* 54:35–38.
20. Jin, T., H. Zhang, G. Boyd, and J. Tang. 2008. Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal death time disks. *J. Food Eng.* 84:608–614.
21. Lu, H., A. E. Castro, K. Pennick, J. Liu, Q. Yang, A. P. Dunn, D. Weinstock, and D. Henzler. 2003. Survival of avian influenza virus H7N2 in SPF chickens and their environment. *Avian Dis.* 47:1015–1021.
22. McLeod, A., N. Morgan, A. Prakash, and J. Hinrichs. 2005. Economic and social impact of avian influenza. Food and Agriculture Organization of the United Nations, Rome.
23. National Agricultural Statistics Services. 2010. Egg products: shell eggs broken. U.S. Department of Agriculture, Washington, DC.
24. Palumbo, M. S., S. M. Beers, S. Bhaduri, and S. A. Palumbo. 1996. Thermal resistance of *Listeria monocytogenes* and *Salmonella* spp. in liquid egg white. *J. Food Prot.* 59:1182–1186.
25. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. *Am. J. Hyg.* 27:493–497.
26. Schuman, J. D., and B. W. Sheldon. 1997. Thermal resistance of *Salmonella* spp. and *Listeria monocytogenes* in liquid egg yolk and egg white. *J. Food Prot.* 60:634–638.
27. Swayne, D. E., and J. R. Beck. 2004. Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathol.* 33:512–518.
28. Swayne, D. E., D. A. Senne, and C. W. Beard. 1998. Avian influenza, p. 150–155. *In* J. Glisson, M. Jackwood, and D. E. Swayne (ed.), A laboratory manual for the isolation and identification of avian pathogens, 4th ed. American Association of Avian Pathologists, University of Pennsylvania, Kennett Square.
29. Tiwari, A., D. P. Patnayak, Y. Chander, M. Parsad, and S. M. Goyal. 2006. Survival of two avian respiratory viruses on porous and nonporous surfaces. *Avian Dis.* 50:284–287.
30. U.S. Department of Agriculture, Food Safety and Inspection Service. 2010. Egg products and food safety, p. 1–4. Available at: http://origin-www.fsis.usda.gov/Fact_Sheets/Egg_Products_Preparation_Fact_Sheets/index.asp. Accessed 13 July 2010.
31. World Health Organization. 2004. Avian influenza A (H5N1) in humans and in poultry in Asia: food safety considerations, p. 1–2. Available at: http://www.who.int/foodsafety/micro/avian1/en/print.html. Accessed 19 March 2010.
32. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus. 2008. Update on avian influenza A (H5N1) virus infection in humans. *N. Engl. J. Med.* 358:261–227.
33. Yamamoto, Y., K. Nakamura, M. Okamatsu, M. Yamada, and M. Mase. 2008. Avian influenza virus (H5N1) replication in feathers of domestic waterfowl. *Emerg. Infect. Dis.* 14:149–151.