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Review

Heat Inactivation of Influenza Viruses—Analysis of Published Data and Estimations for Required Decimal Reduction Times for Different Temperatures and Media

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Abstract: (1) Background: Influenza is a viral infection that has claimed many millions of lives over the past 100 years, and there is always a risk that a new influenza virus will emerge and cause another pandemic. One way to reduce such a potential new influenza virus will be heat inactivation. The question in this study is how much the heat sensitivities of previous influenza viruses differ. If they are very similar, it is expected that a new influenza virus can be inactivated with the same heat parameters as previous influenza viruses. (2) Methods: Through a literature search, published heat inactivation results are compiled and analyzed using Arrhenius models and regression equations for decimal reduction times for different temperatures and media determined. (3) Results: There are about 50 studies on heat inactivation of human and avian influenza viruses so far, showing large differences in heat sensitivity of influenza viruses in different media. However, within a single medium the differences between viruses are rather small. (4) Conclusions: At a temperature of 60 °C, previous influenza viruses can be reduced by 4 or more orders of magnitude within approximately 30 min in almost all media, and this is likely to be true for a potential new influenza virus. Further studies, especially on human influenza viruses, would be desirable.

Keywords: influenza virus; heat; inactivation; decimal reduction time; Arrhenius model

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1. Introduction

The coronavirus pandemic, which began back in 2019, is still ongoing in 2022, and the number of new COVID-19 (Coronavirus Disease 2019) infections worldwide is currently around 1 million per day [1]. Most of this transmission is airborne. Surfaces and other potential carriers seem to play a minor role [2–4].

Influenza is an infection that has many similarities with COVID. The source of severe respiratory illnesses in humans, which can occur in a pandemic, is also an enveloped RNA virus that can also be transmitted via air. Unlike coronaviruses, however, influenza viruses can remain infectious for prolonged periods on surfaces, in liquids, or on other fomites [5–7].

The triggers of human influenza infections are influenza viruses of types A and B, with type A appearing in the form of many subtypes that are distinguished on the basis of their surface proteins hemagglutinin (H1 to H18) and neuraminidase (N1 to N11). For example, the trigger of the largest influenza pandemic to date, the Spanish flu of 1918, with its estimated 50 million deaths, was an influenza A virus of the subtype H1N1 [8]. All subsequent influenza pandemics have also been caused by influenza A viruses [8]. Such a pandemic can be caused by humanity coming into contact for the first time with a new influenza A virus subtype for which no immunity exists in the population. Such a new subtype may appear, for example, when an influenza virus jumps from an animal to a human host. In the past, poultry and pigs have been particularly relevant in this regard, with water fowl considered the natural reservoir of influenza viruses [8,9].

In case of detected influenza infections in humans or animals—it is tried to stop the spread of the virus. For this purpose, it is necessary to inactivate influenza viruses on various fomites. These could be liquids like water, surfaces and animal foodstuffs.

Chemical disinfection and UV radiation are common and effective disinfection techniques, but they cannot always be applied or, in the case of UV radiation for example, may not reach the viruses. Heat inactivation is another well-known disinfection approach that also works in bulk materials. In this process, heat inactivates mainly the relevant viral proteins [10].

There are already some published studies that clearly demonstrate the effect of heat on influenza viruses [11–17]. However, so far, mostly only individual influenza A virus subtypes have been investigated for some contaminated media like phosphate buffered saline (PBS), animal food or filtering facepiece material [18–22]. It would be desirable to be able to make general statements on the temperature sensitivity of all influenza viruses in all relevant media. In case of the emergence of a new pandemic influenza virus, simple heat inactivation protocol suggestions would be already available.

Therefore, in the study presented here, published heat inactivation data for influenza viruses are collected and analyzed to estimate necessary heat application durations for 90% reduction, the so-called decimal reduction time or D value, for different temperatures and media. As a mathematical basis, it is assumed that virus inactivation follows at least approximately an exponential course:

$$c(t) = 10^{-k(T)\ t} \tag{1}$$

c(t) is the concentration of non-inactivated viruses at time t and k(T) is the inactivation rate at temperature T (in Kelvin). In this representation, the reciprocal of the inactivation rate k(T) is equal to the necessary decimal reduction time D(T) for a 90% inactivation:

$$D(T) = \frac{1}{k(T)} \tag{2}$$

In order to compare virus data for different temperatures, a simple model is also applied for the temperature dependence of the inactivation rate, in which it is assumed that the inactivation rate depends exponentially on the temperature T:

$$k(T) = 10^{-\frac{a}{T}+b} \tag{3}$$

$$\rightarrow\ D(T) = 10^{\ \frac{a}{T}-b} \tag{4}$$

$$\rightarrow\ \log(D(T)) = a\ \frac{1}{T} - b \tag{5}$$

with the temperature-independent parameters a and b. In this representation, the logarithm (base 10) of D(T) is a linear function of 1/T.

This approach is the so-called Arrhenius model, which was proposed by Hiatt 1964 [23], among others, and has been successfully applied for a variety of virus inactivation analyses, e.g. influenza viruses [12,24] and also many other viruses [25–31].

2. Materials and Methods

Pubmed and Google Scholar were searched for various combinations of the terms: "influenza", "flu", "heat", "disinfection", "inactivation", "reduction" and "sterilization". Matching publications were examined to determine if they could also be included in this study. In addition, the suitability of all recent publications that cited the previously found sources was reviewed.

When assessing the suitability of studies, only those that addressed the effect of heat of $\geq$40 °C were included. Studies involving lower temperatures or the simultaneous application of other potentially inactivating measures were not considered. Because high

and low pH values can also have an inactivating effect, only studies with mean pH values between 5.5 and 8.5 were included.

The quantitative data required for this analysis were often not directly provided in the retrieved publications. In such cases, it was attempted to determine quantitative values from graphs or tables. For example, in tables of infected or dead chicken embryos, the values for EID50 (embryo infectious dose 50) or ELD50 (embryo lethal dose 50) were determined analogously to the procedure of Reed and Muench [32]. In some cases, the contaminated medium was not explicitly named. In that case, it was assumed that the medium in which the viruses were propagated was also used for the inactivation experiments.

The parameters for the Arrhenius model discussed above were then determined for each medium separately, using linear regression and D(T) was plotted as a linear function of 1/T in each case. With the help of the determined temperature dependence of D(T) for the different contaminated media, expected decimal reduction times for different potential inactivation temperatures are calculated at the end.

3. Results

In total, about 50 publications on heat inactivation were retrieved, the oldest of which is almost 75 years old [11]. The overview of all inactivation data found is given in Table 1. In all cases, influenza virus reduction by the application of heat was observed, but in some cases quantitative analysis was impossible. This was the case, for example, when the temperature changed over the observation period or a virus concentration was below the detection limit after heat application. Where possible, the inactivation duration D for 90% inactivation for the respective virus at the specified temperature is given in Table 1. The publication by Chu mentioned above [11] is the only evaluable study on heat inactivation of influenza B viruses. Otherwise, the results are exclusively for influenza A viruses.

Table 1. Overview of published influenza virus thermal inactivation experiments with virus (subtype), medium and determined decimal reduction time D(T). (* experiments were not included in the quantitative analysis. PBS: phosphate buffered saline, MEM: minimal essential medium, DMEM: Dulbecco's Modified Eagle's Medium, RH: relative humidity).

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in PBS					
H1N1, A/Puerto Rico/8/34, human	56		PBS	successful inactivation but no quantification possible *	[33]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	12.0	PBS		[18]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	4.80	PBS		[18]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	58	2.30	PBS		[18]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	1.30	PBS		[18]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	61	1.00	PBS		[18]
H7N1, A, avian (HPAI)	70	<1.05	PBS	lower detection limit reached; no quantification possible *	[34]
H7N1, A, avian (HPAI)	100	<1.53	PBS	lower detection limit reached; no quantification possible *	[34]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	53	21.7	PBS		[18]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	55	2.80	PBS		[18]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57	2.30	PBS		[18]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	1.20	PBS		[18]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	1.10	PBS		[18]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61	0.80	PBS		[18]
H9N2, A, avian (LPAI)	60		PBS	successful inactivation but no quantification possible *	[35]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in allantoic fluid					
H1N1, A/California/07/2009, human	50	38.46	allantoic fluid (assumed)		[36]
H1N1, A/Beijing/HZ01/2013, human	50	25.00	allantoic fluid (assumed)		[36]
H1N1, A/Puerto Rico/8/34, human	50	27.78	allantoic fluid (assumed)		[36]
H1N1, A/Texas/1/85, human	54	2.31	allantoic fluid (assumed)		[37]
A/Mel (prob. H1N1, A/Melbourne/35), human	56		allantoic fluid	successful inactivation but no quantification possible *	[38]
A/WSN (prob. H1N1, A/WSN/33), human	56		allantoic fluid	successful inactivation but no quantification possible *	[38]
H3N2, A/Aichi/2/84, human	56	4.40	allantoic fluid		[12]
H5N1, A/chicken/Chonburi/Thailand/CU-7/04, avian	55	6.74	allantoic fluid (assumed)		[39]
H5N1, A/chicken/Chonburi/Thailand/CU-7/04, avian	60	6.29	allantoic fluid (assumed)		[39]
H5N1, A/chicken/NakornPatom/Thailand/CU-K2/2004	60	5.15	allantoic fluid (assumed)		[39]
H5N1, A/chicken/NakornPatom/Thailand/CU-K2/2004, avian	65	2.34	allantoic fluid (assumed)		[39]
H5N1, A/chicken/Ratchaburi/Thailand/CU-68/04, avian	55	4.55	allantoic fluid (assumed)		[39]
H5N1, A/chicken/Ratchaburi/Thailand/CU-68/04, avian	60	1.89	allantoic fluid (assumed)		[39]
fowl plaque virus (probably H5N1, A/turkey/Ontario/6213/1966), avian	56	3.57	allantoic fluid		[40]
H7N9, A/Anhui/1/2013, avian	56	1.69	allantoic fluid		[17]
H7N9, A/Anhui/1/2013, avian	65	0.97	allantoic fluid		[17]
H7N9, A/Shanghai/1/2013, avian	56	1.95	allantoic fluid		[17]
H7N9, A/Shanghai/1/2013, avian	65	0.97	allantoic fluid		[17]
H7N9, A/Anhui/1/2013, (human)	50	45.45	allantoic fluid (assumed)		[36]
H9N2, A/chicken/Nanjing/1/2013, avian	50	26.32	allantoic fluid (assumed)		[36]
H9N2, A/turkey/Wisconsin/1966, avian	56	<81.8	allantoic fluid	lower detection limit reached; no quantification possible *	[41]
different influenza A strains	54		allantoic fluid/PBS	successful inactivation but no quantification possible *	[42]
B/Lee, human	50	15.00	allantoic fluid	rough estimation	[11]
B/Lee, human	52	7.50	allantoic fluid	rough estimation	[11]
B/Lee, human	54	3.75	allantoic fluid	rough estimation	[11]
in cell culture medium					
H1N1, A/Netherlands/266/2008, human	56	13.10	DMEM		[43]
H1N1, A/Netherlands/266/2008, human	73	0.53	DMEM		[43]
H1N1, A/NWS/33 (ATCC VR-219), human	70	0.82	DMEM		[14]
H1N1, A/NWS/33 (ATCC VR-219), human	80	0.73	DMEM		[14]
H1N1, A/NWS/33 (ATCC VR-219), human	90	<0.162	DMEM	lower detection limit reached; no quantification possible *	[14]
H1N1, A/Puerto Rico/8/34, human	70	3.33	MEM droplets		[16]
H1N1, A/Puerto Rico/8/34, human	80	1.23	MEM droplets		[16]
H1N1, A/Puerto Rico/8/34, human	90	0.69	MEM droplets		[16]
H1N1, A/Puerto Rico/8/34, human	100	0.50	MEM droplets		[16]
H1N1, A/Puerto Rico/8/34, human	110	0.25	MEM droplets		[16]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in cell culture medium					
H1N1, A/SW/Sk/02, swine	55		MEM	successful inactivation but no quantification possible *	[44]
H3N2, A/Bangkok/1/1979/, human	45	32894.74	DMEM (assumed)	results orders of magnitude above typical results; not included in analysis *	[45]
H3N2, A/Bangkok/1/1979/, human	50	13419.22	DMEM (assumed)	results orders of magnitude above typical results; not included in analysis *	[45]
H3N2, A/Bangkok/1/1979/, human	55	9661.84	DMEM (assumed)	results orders of magnitude above typical results; not included in analysis *	[45]
H3N2, A/Bangkok/1/1979/, human	60	3344.48	DMEM (assumed)	results orders of magnitude above typical results; not included in analysis *	[45]
H3N2, A/Wisconsin/67/2005, human	70		DMEM	successful inactivation but no quantification possible *	[46]
H7N3, A/Mallard/NL/12/00, avian (LPAI)	70		DMEM	successful inactivation but no quantification possible *	[46]
H7N7, A/FPV/Bratislava/79, avian	50	8.33	MEM		[15]
H7N7, A/FPV/Bratislava/79, avian	55	3.70	MEM		[15]
H7N7, A/FPV/Bratislava/79, avian	58	0.75	MEM		[15]
H7N7, A/FPV/Bratislava/79, avian	60	0.53	MEM		[15]
H7N7, A/FPV/Bratislava/79, avian	63	0.38	MEM		[15]
in other liquids					
H1N1, A/NWS/33, human	58	6.71	liquid (blood plasma)		[47]
H5N1, A/NIBRG-14, human	58		liquid (blood plasma)	successful inactivation but no quantification possible *	[48]
H5N1, A, avian (HPAI)	56		peptone water	successful inactivation but no quantification possible *	[49]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	56		different media	successful inactivation but no quantification possible *	[50]
H5N9, A/turkey/Wisconsin/68, avian (LPAI)	56		different media	successful inactivation but no quantification possible *	[50]
H7N3, A, avian	56		peptone water	successful inactivation but no quantification possible *	[51]
H9N2, A/turkey/Wisconsin/66, avian (LPAI)	56		different media	successful inactivation but no quantification possible *	[50]
on surfaces					
H1N1, A/Puerto Rico/8/34, human	55	16.67	stainless steel (surface) RH 25%		[52]
H1N1, A/Puerto Rico/8/34, human	55	5.17	stainless steel (surface) RH 50%		[52]
H1N1, A/Puerto Rico/8/34, human	55	<3.41	stainless steel (surface) RH 75%	successful inactivation but no quantification possible *	[52]
H1N1, A/Puerto Rico/8/34, human	60	12.50	stainless steel (surface) RH 25%		[52]
H1N1, A/Puerto Rico/8/34, human	60	3.66	stainless steel (surface) RH 50%		[52]
H1N1, A/Puerto Rico/8/34, human	60	<2.88	stainless steel (surface) RH 75%	successful inactivation but no quantification possible *	[52]
H1N1, A/Puerto Rico/8/34, human	65	8.33	stainless steel (surface) RH 25%		[52]
H1N1, A/Puerto Rico/8/34, human	65	<2.94	stainless steel (surface) RH 50%	successful inactivation but no quantification possible *	[52]
H1N1, A/Puerto Rico/8/34, human	65	<6.12	different filtering facepiece materials (RH 85%)	lower detection limit reached; no quantification possible *	[22]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
on surfaces					
H1N1, A/WSN/33	105	0.02	surface (steel, polypropylen, cotton)		[53]
H3N2, A/Wisconsin/67/2005 (recombinant), human (?)	72	7.50	droplets on filter material		[54]
H3N2, A/Wisconsin/67/2005 (recombinant), human (?)	82	<6.98	droplets on filter material	successful inactivation but no quantification possible *	[54]
H3N2, A/recombinant strain, human (?)	81	<7.7	towel/filtering facepiece material	lower detection limit reached; no quantification possible *	[55]
H5N1, A, avian (LPAI)	65	<4.31	6 different filtering facepiece materials (RH > 60%)	lower detection limit reached; no quantification possible *	[56]
in chicken meat					
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	57	3.98	chicken thigh meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	57	4.48	chicken breast meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	58	2.17	chicken thigh meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	58	2.56	chicken breast meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	59	1.35	chicken thigh meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	59	1.27	chicken breast meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	60	0.99	chicken thigh meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	60	1.18	chicken breast meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	61	0.48	chicken thigh meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	61	0.57	chicken breast meat		[57]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	57	2.31	breast meat		[58]
H5N1, A/chicken/Korea/ES/2003, avian (HPAI)	30-70		thigh and breast meat	successful inactivation but no quantification possible *	[59]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	2.92	breast meat		[58]
H5N2, A/chicken/Texas/298313/2004, avian (LPAI)	57	2.39	breast meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	4.46	chicken meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	58	2.36	chicken meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	1.36	chicken meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	60	1.06	chicken meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	61	0.39	chicken meat		[58]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	65		thigh and breast meat	successful inactivation but no quantification possible *	[59]
H7N7, A/FPV/Bratislava/79, avian	50	10.71	chicken meat suspension		[15]
H7N7, A/FPV/Bratislava/79, avian	55	3.33	chicken meat suspension		[15]
H7N7, A/FPV/Bratislava/79, avian	58	1.30	chicken meat suspension		[15]
H7N7, A/FPV/Bratislava/79, avian	60	0.48	chicken meat suspension		[15]
H7N7, A/FPV/Bratislava/79, avian	63	0.32	chicken meat suspension		[15]
in egg products					
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	10.73	homogenized whole egg		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	4.48	homogenized whole egg		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	0.37	homogenized whole egg		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	60	0.56	homogenized whole egg		[19]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in egg products					
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	55	6.69	homogenized whole egg		[21]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	57	2.25	homogenized whole egg		[21]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	59	0.36	homogenized whole egg		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	4.28	liquid egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	0.38	liquid egg white		[21]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	55	6.60	liquid egg white		[21]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	57	0.36	liquid egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	3168.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	2016.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	1872.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	61	1440.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	63	288.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	54.4	400.60	dried egg white		[60]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	60	160.70	dried egg white		[60]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	65.5	109.40	dried egg white		[60]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	71.1	43.70	dried egg white		[60]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	55	720.00	dried egg white		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	62.2	0.05	sugared egg yolk		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	63.3	0.02	sugared egg yolk		[19]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	56	1.10	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57	0.53	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	0.44	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	0.39	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	60	0.33	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61	0.31	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	62.2	0.23	sugared egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	63.3	0.13	sugared egg yolk		[20]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	61.1	0.23	fortified egg yolk		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	62.2	0.14	fortified egg yolk		[19]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57	0.91	fortified egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	0.61	fortified egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	0.47	fortified egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	60	0.38	fortified egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61.1	0.13	fortified egg yolk		[20]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	0.34	salted egg yolk		[21]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	62.2	0.06	salted egg yolk		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	63.3	0.04	salted egg yolk		[19]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	0.86	salted egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	0.66	salted egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	60	0.60	salted egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61	0.58	salted egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	62.2	0.50	salted egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	63.3	0.38	salted egg yolk		[20]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	55	0.68	salted egg yolk		[21]
H7N2, A/chicken/New York/13142-5/94, avian (LPAI)	57	0.37	salted egg yolk		[21]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in egg products					
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	60	0.06	plain egg yolk		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	61.1	0.03	plain egg yolk		[19]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57	1.52	plain egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	1.32	plain egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	1.28	plain egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	60	0.73	plain egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61.1	0.67	plain egg yolk		[20]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	62	0.59	plain egg yolk		[20]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	55	18.60	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	56	8.50	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	56.7	3.60	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57	2.50	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57.7	1.10	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	58	0.40	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	0.40	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	55	2.90	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	56.7	1.00	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57	0.80	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	57.7	0.72	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	58	0.60	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	59	0.50	fat free egg product		[61]
H7N2, A/chicken/New York/13142/94, avian (LPAI)	61	0.40	fat free egg product		[61]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	56.7	5.60	egg substitute		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	57.7	2.30	egg substitute		[19]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	59	0.75	egg substitute		[19]
in waste					
H5N1, A/Thai field strain, avian	40		chicken manure	successful inactivation but no quantification possible *	[62]
H5N1, A/chicken/Sikkim/151466/2008, avian (HPAI)	42	1363.64	dry poultry faeces		[63]
H5N1, A/chicken/Sikkim/151466/2008, avian (HPAI)	42	1333.33	wet poultry faeces		[63]
H5N1, rgA/gyrfalcon/WA/41088/2014xPR8, avian (LPAI)	43.3		litter	successful inactivation but no quantification possible *	[64]
H5N1, A/duck/Egypt/VRLCU-R28/2012, avian (HPAI)	56		litter	successful inactivation but no quantification possible *	[65]
H7N1, A/turkey/Italy/4580/1999, avian (HPAI)	43.3		litter	successful inactivation but no quantification possible *	[64]
H7N2, A/chicken/PA/3972-1/97, avian	56	5.15	chicken manure		[66]
H7N2, A/chicken/PA/3972-1/97, avian	60	0.61	chicken manure		[66]
H7N2, A/chicken/PA/3972-2/97, avian	56	8.06	chicken manure		[66]
H7N2, A/chicken/PA/3972-2/97, avian	60	1.48	chicken manure		[66]
H7N2, A/chicken/PA/3779-1/97, avian	56	8.06	chicken manure		[66]
H7N2, A/chicken/PA/3779-1/97, avian	60	1.48	chicken manure		[66]
H7N2, A/chicken/PA/3779-2/97, avian	56	8.63	chicken manure		[66]
H7N2, A/chicken/PA/3779-2/97, avian	60	1.79	chicken manure		[66]
swine influenza	50	29.41	liquid manure		[13]
swine influenza	55	11.76	liquid manure		[13]
H5N2, A/chicken/Pennsylvania/1370/83, avian (HPAI)	>40		compost	successful inactivation but no quantification possible *	[67]

Table 1. *Cont.*

Virus	Temperature [°C]	D [min]	Sample Medium	Remark	Reference
in waste					
H6N2, A/turkey/Mass/3740/65	50–65		compost (different consistence)	successful inactivation but no quantification possible *	[68]
H7N1, A/turkey/Italy/1387/00, avian (HPAI)	45	8.33	compost		[69]
H7N1, A/turkey/Italy/1387/00, avian (HPAI)	45	4.20	compost (different consistence)		[70]
H7N1, A/turkey/Italy/1387/00, avian (HPAI)	45	7.20	compost (different consistence)		[70]
H7N1, A/turkey/Italy/1387/00, avian (HPAI)	55	2.40	compost (different consistence)		[70]
H7N1, A/turkey/Italy/1387/00, avian (HPAI)	55	2.50	compost (different consistence)		[70]

Figure 1 gives an overview of the correlation between 1/T and log(D(T)) for all influenza inactivation results in liquids except liquid animal foods or waste. Log(D(T)) = 0 indicates a decimal reduction time of 1 min, log(D(T)) = 1 is 10 min, and log(D(T)) = −1 represents 0.1 min. Also revealed is the result of a linear regression for D(T). The high scattering or deviation of the individual results from the regression curve is also represented in the relatively low square of the regression coefficient R^2. For the corresponding $D_{lin\ regress}(T)$ from linear regression holds:

$$D_{lin\ regress}(T) = 10^{\frac{3812.6\ K}{T} - 10.995} \quad (6)$$

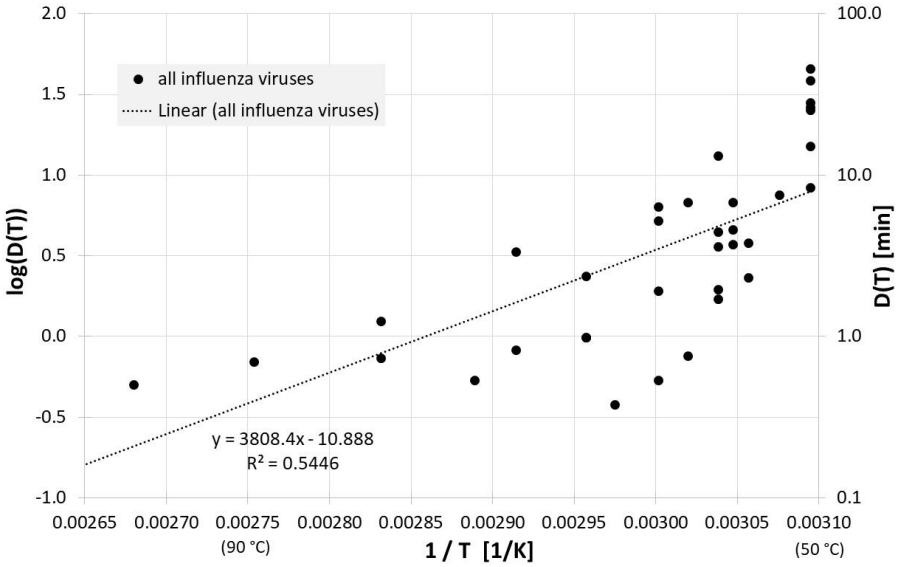

Figure 1. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in liquids other than animal foods or waste along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Due to the scatter of the data, the most often investigated liquids PBS (phosphate buffered saline), allantoic fluid and cell culture medium were also analyzed separately. Figure 2 reveals all results of evaluable inactivation experiments from Figure 1, which were performed in PBS. These are data from Chmielewski et al. [18] for one low pathogenic avian influenza virus subtype (LPAI) and one high pathogenic avian influenza virus subtype (HPAI). Within the limits of their scattering, the values for the two virus subtypes are relatively close to each other and, because of the small data base, no reliable conclusions can be drawn about differences between these viruses. For $D_{lin\ regress}(T)$ the derived formula from the linear regression is:

$$D_{lin\ regress}(T) = 10^{\frac{18183\ K}{T} - 54.596} \quad (7)$$

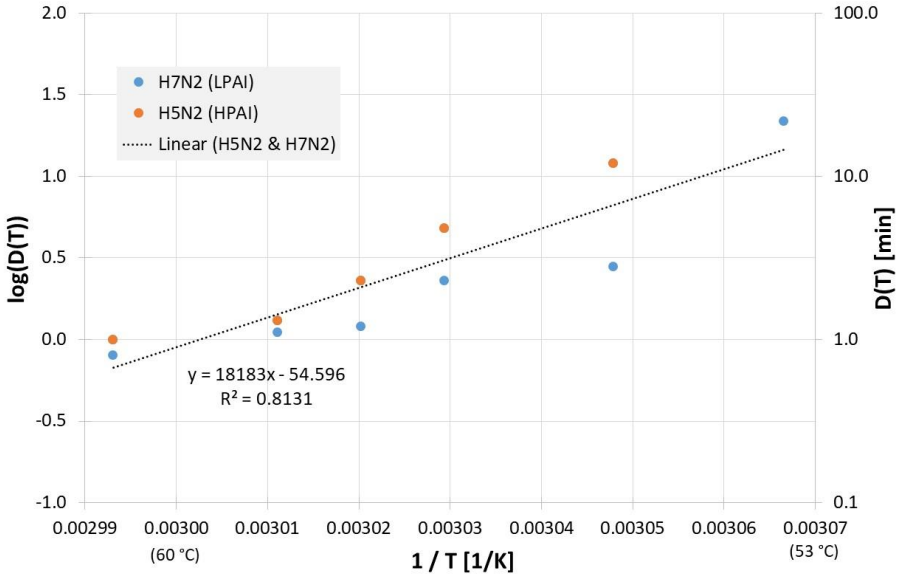

Figure 2. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in PBS along with the linear regression curve and for better understanding, D(T) is also given on the right Y-axis in a logarithmic scale. (LPAI: low pathogenic avian influenza, HPAI: high pathogenic avian influenza).

All results of evaluable inactivation experiments from Figure 1, which were performed in allantoic fluid are presented in Figure 3. Data exist for various influenza A virus subtypes and even for influenza B, but due to the small data base and the scatter of the individual values, no reliable conclusion can be drawn about differences in the temperature sensitivity of these influenza viruses. Based on data of all viruses in Figure 3 the decimal reduction time $D_{lin\ regress}(T)$ is:

$$D_{lin\ regress}(T) = 10^{\frac{8962.1\ K}{T} - 26.512} \quad (8)$$

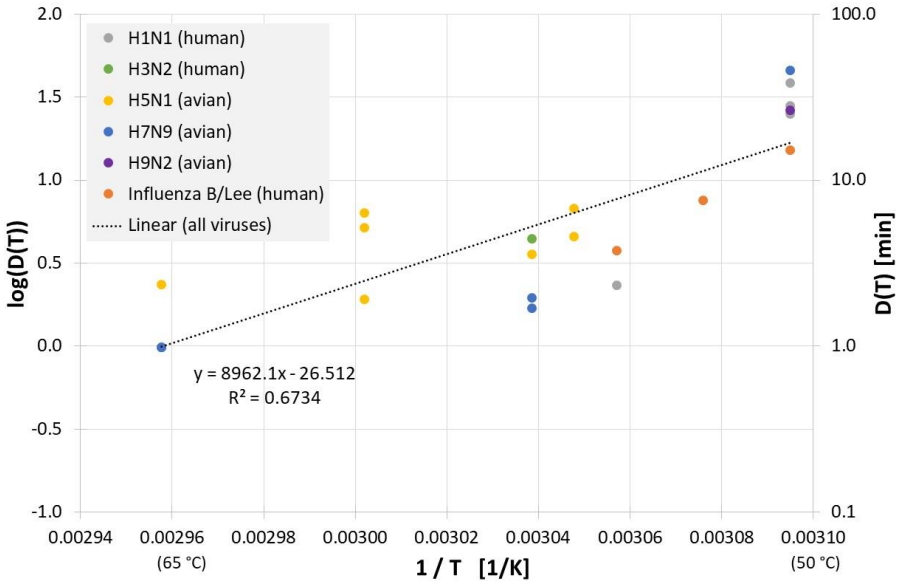

Figure 3. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in allantoic fluid along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Figure 4 shows the results from evaluable inactivation experiments performed in cell culture media. Again, data exist for different influenza A virus subtypes (H1N1 and H7N7), but because the very limited database and different employed cell culture media, no meaningful conclusions can be reached about susceptibility differences between the different influenza viruses. Based on data of all viruses in Figure 4 the decimal reduction time $D_{\text{lin regress}}(T)$ is:

$$D_{lin\ regress}(T) = 10^{\frac{2267.5\ K}{T} - 6.517} \quad (9)$$

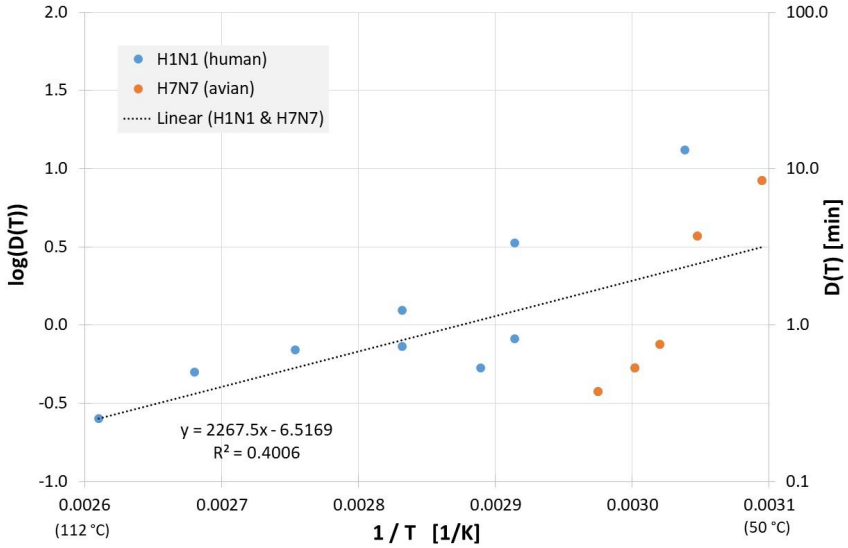

Figure 4. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in cell culture medium along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

The correlation between 1/T and log(D(T)) for all influenza inactivation results on surfaces is presented in Figure 5. For the corresponding $D_{\text{lin regress}}(T)$ from the linear regression is:

$$D_{lin\ regress}(T) = 10^{\frac{6726.1\ K}{T} - 19.279} \quad (10)$$

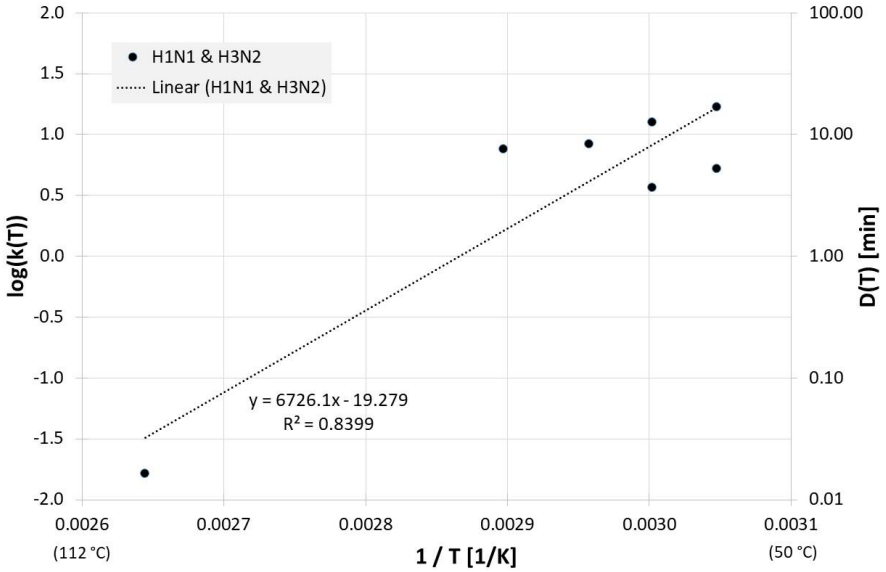

Figure 5. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data on surfaces along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Many published studies deal with the inactivation of influenza viruses in products or waste from the poultry industry. For example, Figure 6 presents the correlation between 1/T and log(D(T)) for all influenza inactivation results in chicken meat. Figure 6 also includes the result of a linear regression for the decimal reduction time $D_{\text{lin regress}}(T)$:

$$D_{lin\ regress}(T) = 10^{\frac{14699\ K}{T} - 44.152} \tag{11}$$

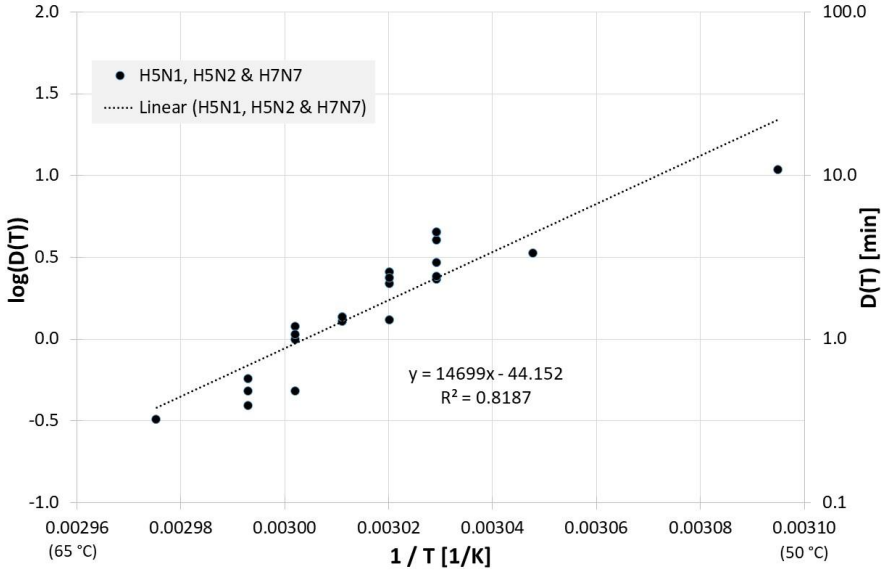

Figure 6. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in chicken meat along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Figure 7 shows the corresponding correlation for homogenized whole egg and the decimal reduction time $D_{\text{lin regress}}(T)$ is:

$$D_{lin\ regress}(T) = 10^{\frac{32006\ K}{T} - 96.587} \tag{12}$$

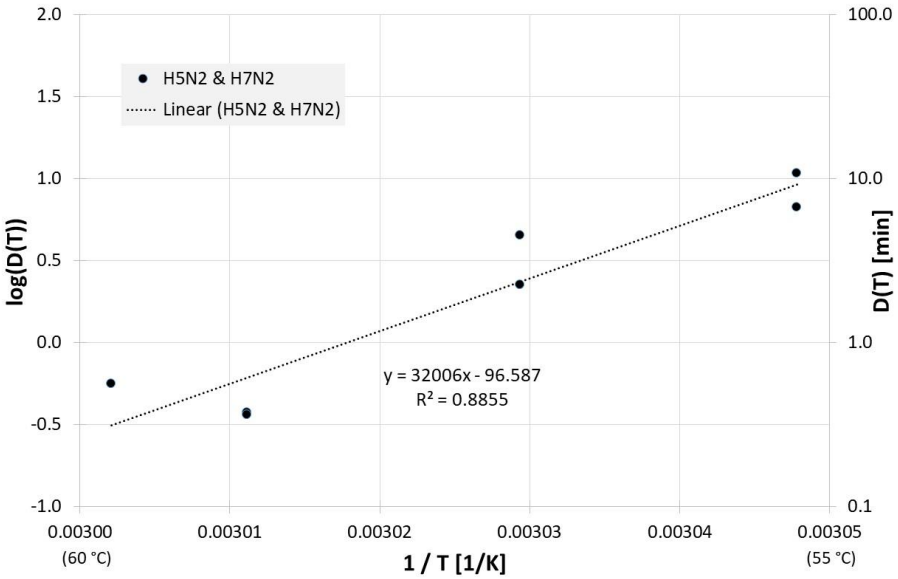

Figure 7. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in homogenized whole egg along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

The published inactivation data for dried egg white can be viewed in Figure 8. For the decimal reduction time $D_{lin\ regress}(T)$ applies here:

$$D_{lin\ regress}(T) = 10^{\frac{9786.4\ K}{T} - 26.674} \quad (13)$$

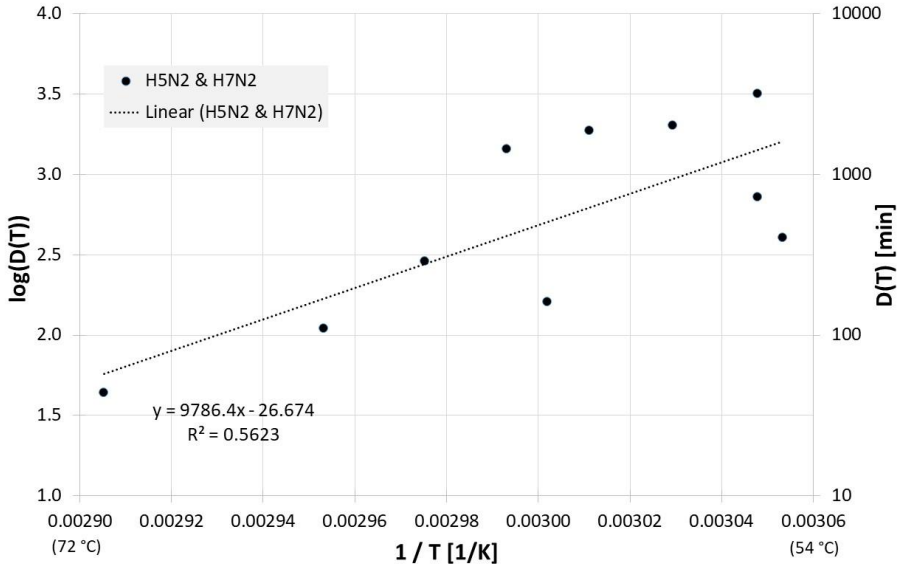

Figure 8. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in dried egg white along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

For plain, salted, sweetened, or fortified egg yolk, the inactivation results published to date can be found in Figure 9, and linear regression of all data yields the following equation for the decimal reduction time $D_{lin\ regress}(T)$:

$$D_{lin\ regress}(T) = 10^{\frac{13098\ K}{T} - 39.814} \quad (14)$$

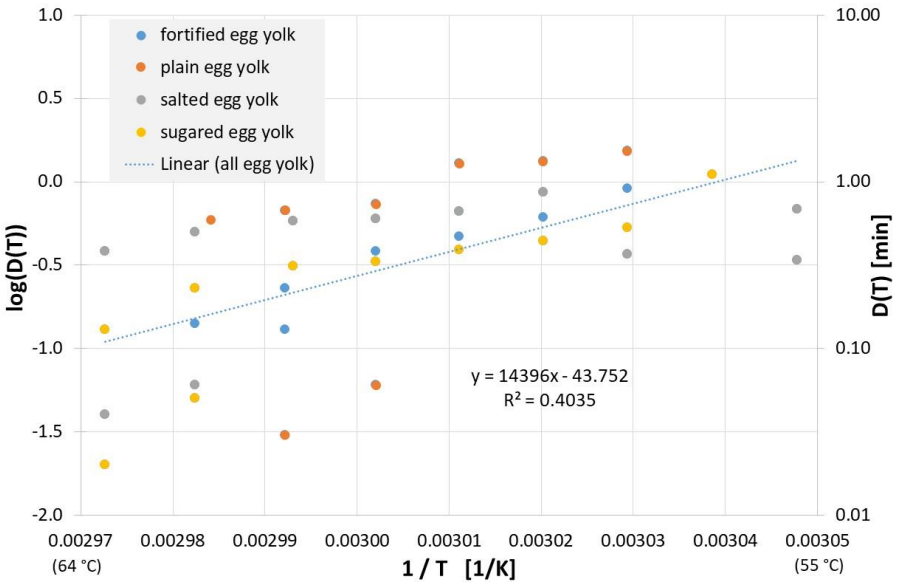

Figure 9. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in plain, sugared, salted and fortified egg yolk along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Inactivation results also exist for fat-free eggs and egg replacements and are depicted in Figure 10, along with the regression line for the decimal reduction time $D_{\text{lin regress}}(T)$:

$$D_{lin\ regress}(T) = 10^{\frac{29081\ K}{T} - 87.814} \quad (15)$$

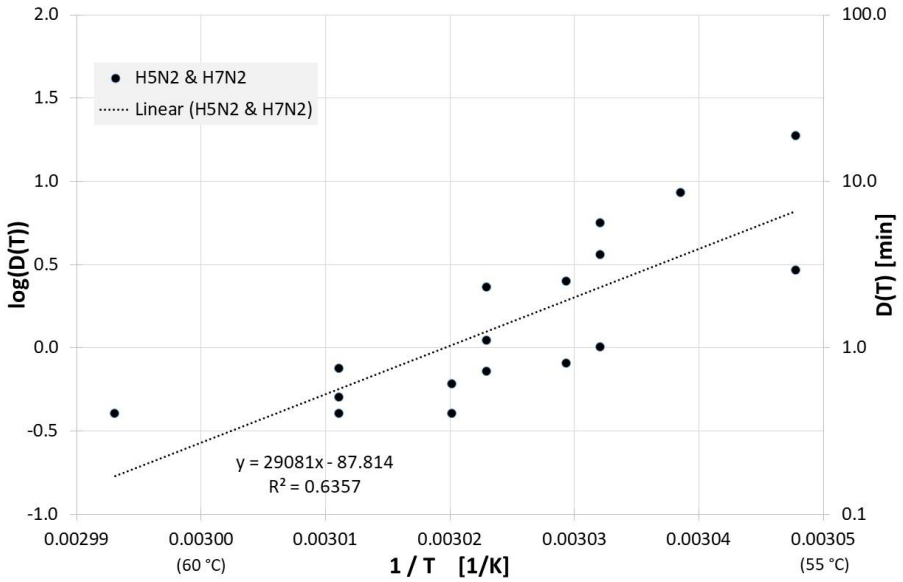

Figure 10. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in in fat free egg and egg substitutes along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

In the event of an influenza outbreak, waste products must also be contaminated. Investigation results for manure and litter can be found in Figure 11. For the decimal reduction time $D_{\text{lin regress}}(T)$ applies:

$$D_{lin\ regress}(T) = 10^{\frac{17227\ K}{T} - 51.567} \quad (16)$$

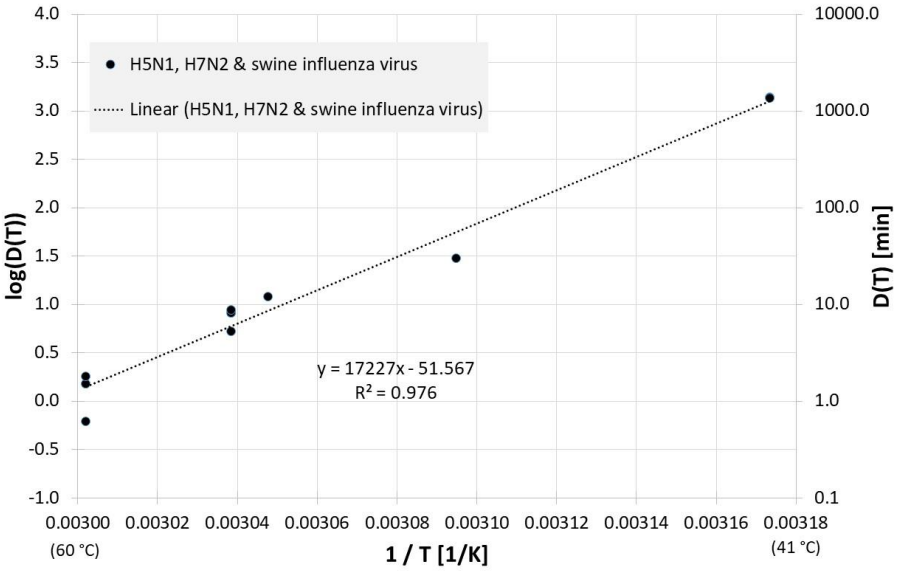

Figure 11. Correlation between 1/T and log(D(T)) for all published influenza virus thermal inactivation data in manure, litter and feces along with the linear regression curve. For better understanding, D(T) is also given on the right Y-axis in a logarithmic scale.

Applying the equations just determined for the decimal reduction times for the various contaminated media, the decimal reduction times for various temperatures were calculated and presented in Table 2.

Table 2. Expected decimal reduction times in minutes for different temperatures and media, determined using the previously determined equations for decimal reduction times.

Medium	Temperature [°C]						
	50	**55**	**60**	**65**	**70**	**75**	**80**
"all liquids"	6.4	4.3	2.85	1.93	1.32	0.91	0.64
PBS	49.9	6.9	1.02	0.158	0.026	0.005	0.001
allantoic fluid	17.2	6.5	2.52	1.007	0.414	0.174	0.075
cell culture medium	3.2	2.5	1.96	1.554	1.241	0.997	0.806
surfaces	35.1	16.9	8.31	4.175	2.141	1.119	0.596
chicken meat	22.7	4.6	0.98	0.217	0.050	0.012	0.003
homog. whole egg	318.3	9.8	0.34	0.013	0.001	<0.001	<0.001
dried egg white	4212	1454	518	190.5	72.07	28.04	11.21
egg yolk	5.5	1.3	0.33	0.087	0.024	0.007	0.002
fat free egg	166.0	7.0	0.33	0.017	0.001	<0.001	<0.001
manure/litter	58.5	9.0	1.47	0.251	0.045	0.009	0.002

4. Discussion

Despite the seemingly large number of individual results, the scope of the data is scarce because the individual results differ not only in temperature, but also in the medium and virus subtype examined. This prevents a more comprehensive investigation for differences between virus subtypes or even for possible differences between avian and human influenza viruses, which could be caused by the different body temperatures with 37 °C for humans and 40–42 °C for birds [9,71].

The small number of available data is also evident, for example, in the investigations in liquid egg white (Figure 8) and compost. For each only 4 and 5 data points, respectively, are available for only two different temperatures. For some of the investigated media, R^2 of the regression curve is only between 0.4 and 0.6, which means that only 40% to 60% of the observed variation of the measured values can be explained by the model (regression curve). It should be noted that the residual variations of 60–40% are probably at least partially due to statistical biological scatter. Additionally, some of the figures display results of different working groups with different equipment and laboratory procedures. E.g., there may be differences in the precision (and speed) with which these different groups were able to adjust and measure the temperature of their virus samples, which would also contribute to the data scattering and a lower R^2.

Inactivation of influenza viruses at comparatively low temperatures starting at about 50 °C is nevertheless clearly evident, but there are major differences with respect to the contaminated media. In particular, in dried egg white, the virus is relatively stable even at high temperatures. For all other media examined, the decimal reduction time from 60 °C is less than 10 min. It should be mentioned that so far, there is no published investigation or even hypothesis on the reason for this obvious dependence of the influenza virus heat stability on the medium.

Antiviral measures officially require a virus reduction of at least 4 powers of ten [72,73]. Under the assumption made above of exponential virus inactivation, an inactivation time of 4 decimal reduction times is necessary for this. With the exception of the dried egg white, such a 99.99% reduction could be accomplished at 60 °C in approximately about half an hour or even faster in almost all media.

These estimates are based on the Arrhenius model and the limited available data. The latter is rather unexpected, since influenza is an infection that typically causes about 500,000 deaths annually [74] and even more in pandemics, up to the estimated 50 million deaths that fell victim to the Spanish flu [75,76]. That is significantly more victims than in the current coronavirus pandemic and yet there are not more studies. In fact, the last

and only study of influenza B virus heat inactivation is even 75 years old and there is also only one quantitative investigation on heat inactivation of influenza A in PBS performed by Swayne and colleagues, who have even generated about half of all single results in this study.

Investigations that are more current are largely concerned with avian influenza viruses. The background does not always seem to be the threat influenza poses to humans, but at least also economic interests of the poultry industry. This is an understandable and comprehensible motivation, but especially against the background of the coronavirus pandemic and the experiences from the influenza pandemics of the last 100 years, one should not wait with further influenza inactivation studies until the next influenza pandemic arrives, which can come at any time [77].

5. Conclusions

It seems that even moderate temperatures around 60 °C, which are well below the 121 °C sterilization temperature commonly used in many areas, are sufficient for influenza virus inactivation within about half an hour. However, the differences in various contaminated media are very large and, at least in dried egg white, influenza viruses are very temperature stable.

Because of the limited data available, it is difficult to determine how large the differences in heat sensitivity are between different influenza virus (sub-)types, or whether there are also relatively heat insensitive influenza viruses and thus whether a future, emerging influenza virus may also be relatively heat stable. Further investigations—prior to the next influenza pandemic—seem reasonable.

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Conflicts of Interest: The authors declare no conflict of interest.

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