INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE MICROBIAL DECAY AND PHYSICAL PROPERTIES OF SHELL EGGS.

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It is well established that shell eggs keep better when stored at low temperature than at moderate or high ones. As a matter of fact the technique of cold storage is based upon this experience. A temperature slightly below zero is found to be quite convenient. As to the marketing of eggs refrigeration is, however, up to now much more an idle wish than an accomplished fact. The way of the eggs from the hen's nest to the consumer's table is in most cases a long and troublesome one and at the different stations of that way: collecting centers, railway-trucks, motor-lorries, and shops, temperature often rises to a level far distant from that of a controlled refrigeration. Though +15°C is recommended as a reasonable temperature for short-time holdings, and higher temperatures are said to be avoided, conditions of a veritable hot climate are temporary not too rare when eggs are transported during summer.

I. Effects at short-holding conditions.

Material and methods.

In order to throw light upon the effect of temperature and humidity on the microbial decay and on the physical properties of white and yolk at short-holding conditions, two series of commercial eggs from the ordinary whole-sale line were subjected to analysis, one of which (I) brought into the whole-sale center at the beginning of March, the other (II) during the first week of May. Eggs were stored at the following conditions:

(I)	High temperature	(+30°C)	Low humidity (40-55% RH)
	Room "	(+20°C)	" "
	Cellar "	(+14°C)	" "
	Refrigeration temperature	(± 0°C)	" "

(II) High temperature (+30°C) High humidity (80-95% RH)
Room " (+20°C) " "
Cellar " (+14°C) " "

All eggs were clean, medium size, candling grade AA-A, laying-time and origin not known. Age of eggs at the beginning of storage was supposed to be about one week. Samples were collected and 100 eggs broken for control of original quality. This was accomplished by means of the following measurements:

1. Height of thick albumen
2. Height (h) and width (w) of yolk ($\frac{h}{w}$ = yolk index)
3. pH of albumen

The first two operations were carried out with the aid of a tripol-micrometer similar to that used by Wilgus & van Wagenen (1935). As all eggs were weighed on an automatically operating balance with fairly good accuracy (0,1 g) it was possible to compute Haugh units for all eggs according to Brant & Morris (1951) from the formula:

$$\text{Haugh unit} = 100 \log \left[H - \frac{\sqrt{G(30\ W^{0.37} - 100)}}{100} + 1{,}9 \right]$$

where H denotes the height of thick albumen (mm), W the weight of the egg (g) and where G = 32,2. Haugh units can within reasonable limits be calculated directly by the aid of a special slide-rule calculator (according to Brant & Morris l.c.). Eggs were divided into the following grades and scores:

Grade:	AA	A	B	C
Scores:	1 2 3	4 5 6	7 8 9	10 11 12
Haugh units:	100-78,2	78,1-55,2	55,1-31,2	31,1-0

Results obtained by the different measurements were compared to the impression derived from a chart of photographs (Brant & Morris l.c.) selected as typical of the 12 scores of the 4 grades and including the entire scale of Haugh units. These observations were found to be in fairly good accordance with the figures obtained by measurement.

Original grade

From samples taken at the beginning of storage the following figures were computed:

Grade:	(I.)	(II.)
AA-A (scores 1-6)	85%	45%
B (scores 7-9)	8%	47%
C (scores 10-12)	7%	8%
mean (P=95%) grade:	$5,37 \pm 0,29$	$7,09 \pm 0,39$
pH:		
of albumen	8,9	9,3

Results

Microbial decay

Fig. I. gives an instructive picture of the microbiological situation during storage. It is of course not possible to predicte expected figures for microbial loss at different points of time under various conditions, because this loss to a great degree depends on other factors than temperature and humidity, e.g. severity of microbial attacks prior to storage, hygienic conditions of storage room, equipment, cases, fillers, and flats. The diagram only points out what may occur at certain temperatures and humidities under conditions similar to those prevailing in this investigation.

At high temperature and high humidity (30°H) the course of deterioration was dramatic. After 3 days of latency, figures for microbial decay rose to such a level that after 4 more days 73% of eggs were found to be spoiled by attacks of molds and bacteria. At high temperature and low humidity (30 L) the latent period was 6 days. First after 3 weeks a severe increase was noted. 30% of eggs were deteriorated after 21 days and 36% after 37 days.

At room temperature, humid air conditions, (20°H) only a few rots were found after 17 days, though all eggs were covered with mold. Then deterioration went on rapidly. Nearly 57% of eggs were lost after 23 days in storage. At room temperature, low humidity, (20°L) the latent period was highly extended. Only 2 rots were found until the 22nd day, and after 62 days in storage 10% of eggs were

heavily infected.

At cellar temperature, high relative humidity (14°H) rots were found only occasionally during the first month. After 45 days in storage slightly more than 15% were spoiled. Green rots are now predominating though mold still plays an important rôle. At cellar temperature, low relative humidity (14°L) the first rots were found after 27 days. Here, too, the green rots were predominating.

At refrigeration conditions (0°H) no microbial deterioration was noted.

Interior quality

Decreasing mean grade

Fig. II a, b, and Tab. 1, a, b show the decreasing tendency of the mean score originally found. It is to be noted that this value was different in the two series (I) giving medium grade A (scores $5{,}37 \pm 0{,}39$) and (II) giving high grade B (score $7{,}09 \pm 0{,}39$).

For eggs stored at 30°C, low humidity, this mean value rapidly passed grade A and ran into grade B within 1 - 2 days; the B-C grade limit was crossed within 6 days.

At 20°C the A-B line was crossed at about the same time as the mean of eggs at 30°C passed the B-C line (6 days).

At 14°C the former limit is crossed within 10 days, but it takes about 25 days to reach the limit between grade B and grade C.

At 0°C the mean score remains within the limits of grade A still after 1 month of storage.

At high humidity the tendency was about the same as shown by fig. II b. The mean score rapidly runs downward, reaching the B-C limit within 3-4 days. The curves for +20°C and +14°C seem to run more close together, the latter remaining inside grade B for a reasonable time (about 26 days).

Change in physical properties of white and yolk

Changes in Haugh units, yolk index and pH value of the white followed the same tendency as that of the mean scores.
The rapid decrease of Haugh units as well as yolk index at 30°C

is fairly well demonstrated. Noteworthy is the change in pH (Almgren, 1952). As expected these values increase during the first time of storage. Later on, however, they tend to decrease the sooner the higher is the temperature. This is quite in agreement with the findings mentioned by Romanoff & Romanoff (1949) but opposed to those of Sharp (1929). This change observed at many occasions when eggs are stored at high temperatures seems to stress the opinion that pH of the white is an unsuitable value as a measure of interior egg quality.

Conclusions

Temperature in the range of 30°C is inexorably to be avoided, or the interior quality will be lowered extremely rapidly and micro-organisms may make great havoc especially when the relative humidity is high. At short-time holding using temperature in the range of +20°C and +14°C (to be preferred) quality will remain within reasonable limits. When higher temperatures are unavoidable, eggs are to be kept at medium dry conditions, the higher range of relative humidities only being used when refrigeration is possible.

II. Effect of heat treatment on microbial decay, interior quality and quality retention during subsequent storage at various conditions

Trying to improve the possibility of eggs to withstand the strain of various conditions the following measures were taken:

(1) oiling - to reduce gas exchange and subsequent weight loss

(2) evacuation and stabilization with CO_2 - to reduce O_2 and stabilize the properties of albumen

(3) treatment of shell surface with various compounds - to get the microbiological situation within control.

The result of these efforts (Almgren, 1953, 1954) shows that certain improvements can be obtained. Thus after cold storage for 6 1/2 months ($\pm$ 0°C, about 50% RH) 50% more grade A eggs were received when eggs were oil treated and 200% more eggs of this high grade when eggs were oiled in connection with evacuation and CO_2-

stabilization. The microbial situation, however, still remains unsatisfactory. To meet this difficulty two ways were open:

(A) Treatment as to point (3). mentioned above, and

(B) Heat treatment (pasteurization).

Control of microorganisms by adding chemicals with germicidal properties was attempted in experiments including quarternary ammonium compounds, the results, however, not being quite conclusive. A study of the successful results from investigations on heat treatment of eggs in USA (Funk, 1943, 1950, Goresline et al 1950,1952) and in Australia (Murphy et al 1947, Salton et al 1951) indicated that pasteurization was worthy to be investigated before any further experiments with germicides is carried out.

Heat treatment

Vacuum-CO_2-heat treatment in oil

The following method was applied (Almgren 1953) in experiments of laboratory scale:

Two glass exsiccators were connected with each other and with a vacuum pump and a CO_2-tube, one for oil and the other for eggs to be treated. Vacuum is applied (1) and pressure re-established by CO_2 (2). Then oil is let into the exsiccator with eggs (2 b) and vacuum is applied again (3). After outlet of oil (3 b) eggs remain in CO_2 under atmosphere pressure until treatment is finished (4).

Duration of the four steps:

(1)	Evacuation (300 mm Hg)	150 sec.
(2)	Re-establishment of atmospheric pressure by CO_2	150 "
(2b)	Supply of oil	15 "
(3)	Evacuation + oil (300 mm Hg)	300 "
(3b)	Outlet of oil	15 "
(4)	Re-establishment of atmospheric pressure by CO_2	300 "
		930 sec.
		(15 1/2 min.)

During this treatment two cisterns, a and b, were placed in a waterbath with electric water heating and a contact thermometer as well as a stirrer, enabling the oil to remain at a constant temperature. Inside the cistern, thermometers with a measuring field of between +60 and +70°C were placed. This temperature of the oil had to be adjusted according to the number of eggs and also considering the temperature inside the egg at the beginning of the heat treatment, so that the highest possible effect was attained during the time available (300 sec.). If the oil was kept at a temperature of +70°C in the oil cistern, it dropped to +64°C after the treatment, when 30 eggs, holding room temperature had been thermostabilized. Thus the limit for the permissible heat-treatment had been passed. This was proved by small spots of coagulated albumen, which were visible at about 10% of the eggs, but from a practical point of view were of less importance. With the assistance of thermoelements (copper-constantan) - the thermocouples could be placed in the egg in appropriate capillary tubes from glass closed to the yolk membrane - it was possible to follow up the temperature inside the egg during the course of the procedure. From these measurements it was evident:

1. that the temperature inside the egg near the yolk membrane is rising during the whole procedure
2. that a striking increase of temperature occurs during moment (3), (vacuum-oil)
3. that the temperature continues to rise during the CO_2-equalization at next moment, and
4. that under conditions here prevalent +54°C is the highest temperature tolerated by the albumen near the yolk membrane without the occurence of partial coagulation.

Pasteurization in oil

For this heat treatment eggs were put into the cistern. Hot oil (+70,3°C) was taken in. The procedure lasted during 10 min. After the oil had been removed, the temperature dropped to +63,1°. No coagulation-spots occurred in albumen, but this was the case at an initial temp.

of +71°C and a final temperature of +64,5°C. A diagrame showed the course of the temperature during these two procedures, and delimited the highest applicable temperature. As has been proved before, the albumen near the yolk membrane then attains a temperature of +54°C. During the vacuum-CO_2-oil heating procedure the same result was achieved only after 5 minutes of treatment in oil. The egg temperature is then, however, affected by the other moments of the procedure.

During a couple of years about 6000 eggs were subjected to analysis in comparative experiments, where different kinds of treatment were applied. Thus vac.-CO_2-oil-pasteurized eggs were compared to eggs pasteurized in oil and to vac.-CO_2-oiled, oiled, and untreated eggs.

Microbial decay and changes in quality was determined according to methods mentioned in part I of this paper.

The results will be clear from tab. 2-7.

Tab. 2 shows the microbial loss from different experimental series. The low figures for pasteurized eggs are to be noticed.

The quality retention will be clear from tab. 3, showing the remaining % of grade A eggs calculated from the original % of this quality. More than 100% retention is obtained in one case (110%). This increase is shown to be the result of a direct improvement.

Tab. 4-5 give the figures from two series for pH, Haugh units, yolk index, weight loss, and distribution into grades and scores, tab. 4 at room conditions, and tab. 5 at cold storage conditions. The improvement one day after treatment is well demonstrated by the latter table.

Conclusion

Through combining various treatments, aiming at improving the interior quality of eggs, substantial improvements have been attained. After storing for 6 weeks at +20°C and about 40% RH, the percentage of eggs, belonging to grades AA and A, was still unchanged.

Through oil dipping only a slight improvement of the keeping quality was registered, being more accentuated with length of storage time. If oiling was complemented by exchanging the gas in the air chamber by removing a great part of the oxygen and adding carbon dioxide, the preservative effect was still more enhanced. After 9 weeks storage this treatment showed six times higher preserving effect compared to an exclusive oiling-treatment. If the vac.-CO_2-oil procedure was combined with pasteurization so that the oil was heated to a defined temperature, a still more striking increase in the stabilization of the egg quality was achieved. The eggs maintained their quality unchanges for 6 weeks. Even after 9 weeks 90% of the eggs remained in the A quality class.

When in another experimental series the vac.-CO_2-pasteurization in oil was compared to pasteurization in oil at defined conditions, both methods gave eggs with high quality retention. Besides the preservative effect a real improvement was found. After cold storage at +1 - +3°C, 70% RH for 6 - 9 months the quality retention was increased by the various treatments. In trials where pasteurization was applied, up to 90% of the original quality was retained. Untreated eggs showed here at the best 10%. Oiled and vac.-CO_2-oiled eggs gave up to 25% retention of quality at the same conditions.

From microbiological point of view it was possible to establish that eggs could be stored for 6 weeks at +20°C and about 40% RH without microbial losses after treatment according to the methods here mentioned. Slight losses were found after 15 - 18 weeks' storage but no case of green rot.

After cold storage at +1 - +3°C, 70% RH for 6 - 9 months, vac.-CO_2-oil pasteurized eggs gave not more than 2 - 2,5% of rots, compared to 12 - 15% of untreated samples.

It is further remarkable that, instead of being harmful, the low RH of the storage atmosphere at room temperature turns out to be an asset under the conditions, applied in this procedure.

Parts of this paper have been presented by the director of the Institute at the Conference on storage of eggs in Cambridge 6 - 11 April, 1956 arranged by the International Institute of Refrigeration.

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Tab. 1 a

Mean grade after 0 - 28 days in storage at 40-55% relative humidity

Days in stor.	+30 °C		+20 °C		+14 °C		±0 °C	
	m x)	sd xx)	m x)	sd xx)	m x)	sd xx)	m x)	sd xx)
0	5,37±0,39	±1,96	5,37±0,39	±1,96	5,37±0,39	±1,96	5,37±0,39	±1,96
4	7,99±0,29	±1,49						
5			6,18±0,29	±1,48				
6	9,83±0,29	±1,52						
7					5,58±0,37	±1,93		
8	11,32±0,27	±1,35						
11			7,97±0,29	± 1,53				
12					6,94±0,35	±1,79		
14							6,24±0,39	±2,02
19			8,24±0,27	±1,37				
20					7,10±0,33	±1,74		
26			9,74±0,27	±1,32				
27					8,10±0,33	±1,66		
28							5,89±0,24	±1,24

x) mean (P = 95%)

xx) standard deviation

Tab. 1 b

Mean grade after 0 - 30 days in storage at 80 - 95% relative humidity

Days in storage	+30°C		+20°C		+14°C	
	m x)	sd xx)	m x)	sd xx)	m x)	sd xx)
0	7,09±0,39	±1,98	7,09±0,39	±1,98	7,09±0,39	±1.98
2	7,67±0,32	±1,55				
4			7,51±0,32	±1,55		
7	11,25±0,18	±0,88				
10			8,51±0,29	±1.52		
14					8,63±0,43	±2,24
17			8,55±0,32	±1,62		
24					9,08±0,27	±1,37
30					10,13±0,33	±1,66

x) mean (P = 95%)

xx) standard deviation

Grade 1 - 3 = AA

" 4 - 6 = A

Grade 7 - 9 = B

" 10 - 12 = C

Tab. 2

Microbial decay (% rots)

(From different experiments at various conditions)

Time and conditions of storage	No treat-ment	Oil	Vac.-CO_2-oil.	Vac.-CO_2-past.in oil	Past. in oil
6 weeks (+20°C, 40-50% RH)	---	---	0	0	0
12 " -"-	7	---	---	1,5	---
12 " -"-	5,5	---	---	---	0,7
15 " -"-	4	---	---	0,5	0
18 " -"-	6	4	2	1,5	---
6 1/2 months (+0°C,50% RH)	12	12	17,5	---	---
9 months (+1-+3°C, 70%RH)	15	15	13	2,5	4
9 " -"-	12	---	---	2	0

Tab. 3

Remaining % of original quality

(Calculated as remaining % grade A)

(From different experiments at various conditions)

Time and conditions of storage	No treat-ment	Oil	Vac.-CO_2-oil.	Vac.-CO_2 past.in oil	Past. in oil
3 weeks (+20°C, 40-50% RH)	10	---	---	90	95
3 " -"-	30	50	70	100	--
3 " -"-	40	---	---	110	95
6 " -"-	0	---	---	65	85
6 " -"-	10	30	75	100	75
6 " -"-	0	---	---	85	---
9 " -"-	0	---	---	60	70
9 " -"-	0	10	60	90	80
9 " -"-	0	---	---	85	---
6 months (+1-+3°C, 70% RH)	10	25	25	90	90
6 " -"-	10	---	---	70	70
9 " -"-	0	0	20	50	70
9 " -"-	10	---	---	60	55

Tab. 4

Storage conditions Temp.: +20°C RH.: 40-50%	Original value:	No treatment weeks in storage			Past. in oil weeks in storage			Vac.-CO_2-past. weeks in storage		
		3	6	9	3	6	9	3	6	9
pH /albumen/	9,19	9,50	9,55	9,54	9,34	9,42	9,46	9.25	9.27	9.34
Haugh value	75	52	30	25	76	71	66	76	67	66
Yolk index	0,41	0,38	0,31	0,26	0,39	0,36	0,34	0,41	0,37	0,35
Weight loss %	---	4,6	10,7	15,5	0,4	1,5	1,5	0,3	0,6	1,2
Grade AA (1-3)[1]%	26	0	0	0	36	3	10	33	10	6
" A (4-6)[1]%	73	41	3	0	53	67	63	67	70	74
" B (7-9)[1]%	7	34	34	25	11	30	19	0	20	20
" C (10-12)[1]%	0	25	63	75	0	0		0	0	0

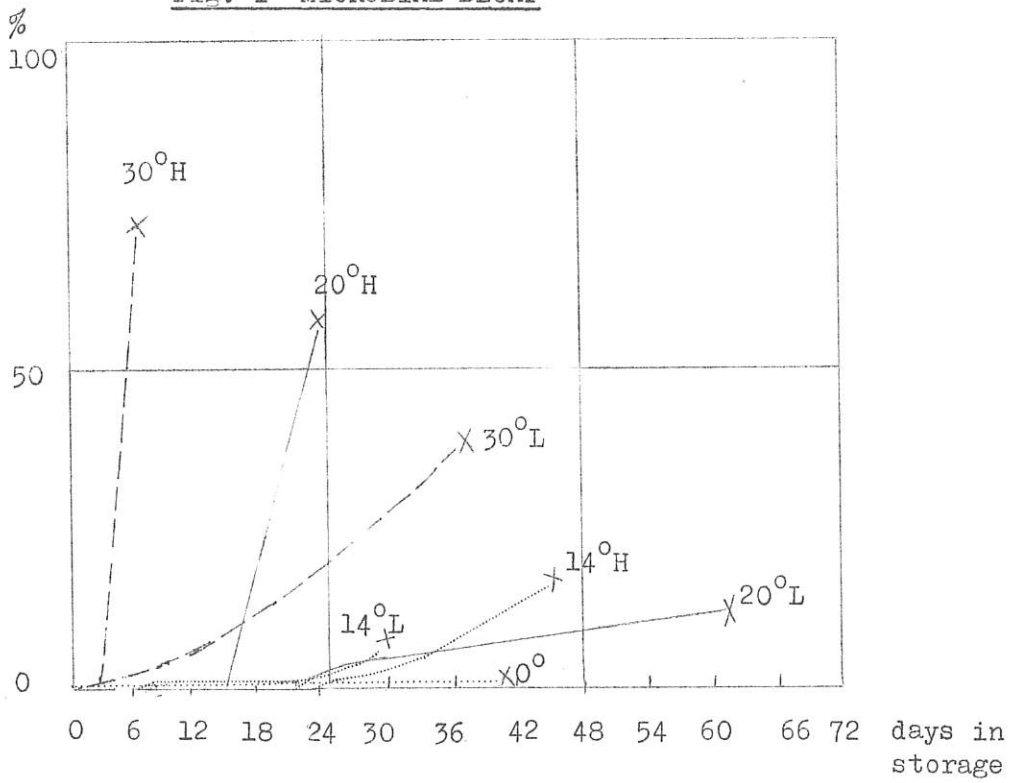
1) Scores according to Brown & Morris, 1951

Tab. 5

	No treatment			Vac.-CO_2-past. in oil			Past. in oil		
	Before cold storage	After 6 months	After 9 months	Before cold storage[2]	After 6 months	After 9 months	Before cold storage[2]	After 6 months	After 9 months
ph of albumen	9,35	9,26	9,17	9,29	8,92	8,85	9,11	8,79	8,81
Haugh units	54	38	37	68	58	57	67	57	56
Yolk index	0,39	0,36	0,35	0,37	0,36	0,35	0,40	0,36	0,35
Weight loss	---	8,0	12,4	---	1,7	3,1	---	1,7	2,7
Grade AA (1-3)[1] %	2	0	0	18	3	2	15	1	2
Grade A (4-6)[1] %	48	5	6	64	52	43	68	58	46
Grade B (7-9)[1] %	38	51	33	18	40	50	15	34	48
Grade C (10-12)[1] %	12	44	61	0	5	5	2	7	4

1. Scores according to Brant Morris
2. 1 day after treatment

Fig. I MICROBIAL DECAY



$30^{\circ}H$ = $+30^{\circ}C$,	80-95%	rel. humidity
$30^{\circ}L$ = $+30^{\circ}C$,	40-95%	-"-
$20^{\circ}H$ = $+20^{\circ}C$,	80-95%	-"-
$20^{\circ}L$ = $+20^{\circ}C$,	40-55%	-"-
$14^{\circ}H$ = $+14^{\circ}C$,	80-95%	-"-
$14^{\circ}L$ = $+14^{\circ}C$,	40-55%	-"-
0° = $\pm 0^{\circ}C$,	40-55%	-"-

Fig. II A 40 - 55 % Relative humidity

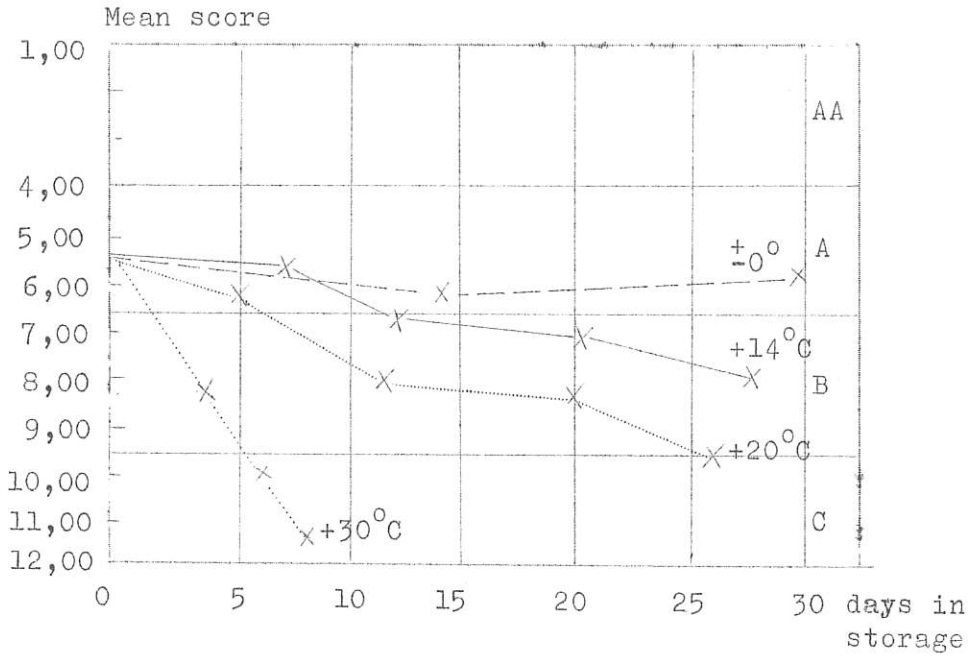


Fig. II B 80 - 95 % Relative humidity