Bread Staling Studies. II. The Role of Refreshening[1]

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ABSTRACT

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Refreshening studies of breads with and without surfactants were conducted. Breads stored at 2 and 30° C were refreshened by heating at 90° C for 45 min. Those stored at 2° C for two days and refreshened regained the original firmness. Breads refreshened two and three times, however, did not revert to their original firmness. Refreshening of breads kept at 30° C resulted in only partial recovery of the firmness of fresh bread. Surfactants (sodium stearoyl lactylate and Atmul 500) were helpful in restoring original freshness. Breads kept at 2° C for two and four days could be restored to original firmness by first and second refreshenings; at 30° C, original firmness was only partially recovered. Firmness data are supported by organoleptic tests, X-ray diffraction patterns, and soluble starch data.

Numerous studies have been done on the refreshening of starch gels, gluten gels, and breads. Using differential thermal analysis, Colwell et al (1969) observed the disappearance of the endothermic peaks at 71 and 68° C for starch gels retrograded at 35 and 30° C, respectively. These changes in the thermograms indicated that the starch gels were restored to their original order. Whereas retrogradation of amylose is not reversed by heat, retrograded amylopectin reverts to its amorphous state when energy equivalent to a 40–50° C temperature increase is applied (Schoch and French 1947). This implicates amylopectin as a major factor in bread staling. Cluskey et al (1959) and Willhoft (1971, 1973) reported that, unlike starch retrogradation in bread crumb, protein denaturation and moisture redistribution cannot be reversed by heating. Zobel (1973) noted that the X-ray diffraction pattern of stale bread reverts to that of a freshly baked bread when the bread, in a moist state, is heated at 95° C.

The purpose of this study was to investigate the effects of heat-refreshening on breads produced with and without surfactants. These effects were estimated by changes in crumb firmness, content and composition of soluble starch, X-ray diffraction patterns, and sensory perception of freshness. The repeatability of refreshening was also studied.

MATERIALS AND METHODS

Flour Sample

A straight grade, totally untreated flour milled on a Miag pilot mill was used (Shuey and Gilles 1969). Its protein, ash, and farinograph absorption values were 13.0, 0.43, and 64.5%, respectively, on a 14.0% moisture basis.

Baking Additives

Sodium stearoyl-2-lactylate (Emplex) was obtained from Patco Products Division, C. J. Patterson Co., Kansas City, MO. Atmul 500, a soft plastic form of mono- and diglycerides, was obtained from ICI Americas, Wilmington, DE.

Bread Samples

The baking procedure and formula reported previously (Pisesookbunterng and D'Appolonia 1983) were used in making 3,000 g of flour into control, control + sodium stearoyl lactylate (SSL) (0.5%, flour weight), and control + Atmul 500 (0.5%, flour weight) breads.

Nine loaves were baked from 3,000 g of flour, cooled for 2 hr, and sliced. Firmness on one loaf was measured immediately. Eight loaves were wrapped in tin foil and stored at 2 or 30° C and 80% relative humidity. After four days, one loaf was removed from storage, left at room temperature for 3 hr, and its firmness measured. After six days total storage time, another loaf was removed, allowed to sit 3 hr at room temperature, and its firmness measured.

The remaining six loaves were removed from storage after two days and left to sit at room temperature for 3 hr. Firmness on one loaf was measured immediately. The other five loaves were refreshened at 90° C for 45 min. After one loaf was allowed to sit at room temperature for 2 hr, its firmness was measured. The remaining four loaves were returned to correct storage conditions. These were removed from storage after an additional two days (four days total), and allowed to sit 3 hr at room temperature. Firmness on one loaf was then measured. The remaining three loaves were refreshened a second time at 90° C for 45 min. Firmness on one of these loaves was measured after it was allowed to sit at room temperature for 2 hr. The final two loaves, after a total of two refreshenings, were returned to storage for an additional two days. After the six days total storage time, they were removed and allowed to sit 3 hr at room temperature. Firmness on one loaf was then measured. The final loaf was refreshened a third time at 90° C for 45 min. Firmness, after the bread sat 2 hr at room temperature, was then measured.

The breads, wrapped in tin foil, were stored at 2 or 30° C and 80% rh, then refreshened by heating at 90° C for 45 min.

Firmness Values

Firmness of bread crumb was measured on bread slices with an Instron Universal Testing Instrument (Instron Corporation, Canton, MA).

The bread slices were compressed for a distance of 0.5 cm with a specially designed Plexiglas™ tooth described by Walsh (1971). Three measurements were made on each of three slices of bread, and the results were averaged. Slices from the same position in each loaf were used for all measurements. These included the slice in the center of the loaf and the slice adjacent to the center on each side. The crust on the bread slices was left intact during firmness measurements.

Soluble Starch, Amylose, and Amylopectin Content

Bread slices used for the firmness measurement were decrusted, freeze-dried, and ground on a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 40-mesh sieve. Contents and compositions of soluble starch in the bread crumb were then determined by a procedure used by Pisesookbunterng and D'Appolonia (1983).

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Sensory Evaluation

Sensory evaluation of bread freshness was investigated using a triangle test. Ten panel members were selected from a group of 15 on the basis of their ability to discern differences in staleness. In actual testing, the panel was given three lots of three slices of bread and instructed to select the odd sample. The comparisons made were stale vs fresh, fresh vs two-day stale, and two-day stale vs refreshened two-day stale breads. Because traces of incipient mold appeared in bread stored beyond four days at 30°C, sensory evaluation was not conducted on bread stored for an extended period.

X-Ray Diffraction Studies

X-ray diffraction studies were performed on the freeze-dried bread crumbs that contained no additives and were used in refreshening. The samples examined were those from which firmness values were obtained before freeze-drying and those in which soluble starch content was measured after drying. The X-ray diffraction method was that described by Dragsdorf and Varriano-Marston (1980).

RESULTS AND DISCUSSION

Firmness Values

Breads Without Surfactants. Table I shows the firmness values of breads stored at 2 and 30°C for two, four, and six days, before and after freshening. The crumb firmness of all breads kept at 2°C for two days could be essentially restored to the original values by refreshening. This conclusion is supported by statistical treatment of the data (Table II). These results suggest that at 2°C the heat-reversible character of the starch plays a major role in controlling the firming process.

When the breads stored at 2°C were refreshened for the second or third time, they failed to revert to their original firmness. Moisture loss by evaporation and/or redistribution between crumb and crust may have occurred. In addition, increases in protein denaturation which are not heat-reversible may have contributed to decreased reversibility of crumb firmness after longer storage at 2°C.

Breads stored at 30°C and refreshened did not regain their original freshness. Subsequent second and third refreshenings were also only partially effective (Tables I and II). These trends suggest that factors other than starch retrogradation are involved in the

TABLE I
Refreshening of Bread: Firmness Values[a]

Storage Time (Days)	Refreshening Process	Bread: Control 2°C	Control 30°C	With SSL 2°C	With SSL 30°C	With Atmul 500 2°C	With Atmul 500 30°C
0[b]	...	82	84	96	82	75	75
2	Before first refreshening	393	233	331	176	264	137
	After first refreshening	99	146	97	125	83	117
4	No refreshening	459	277	393	216	339	234
	After first refreshening and before second refreshening	432	285	294	234	269	187
	After second refreshening	119	180	100	165	74	140
6	No refreshening	516	345	411	263	395	306
	After second refreshening and before third refreshening	391	315	312	245	289	226
	After third refreshening	124	258	120	198	104	180

[a] Values are expressed as g·cm and represent an average of nine measurements—three determinations on each of three slices of bread.
[b] Zero-day storage represents bread after 2 hr removal from oven.

TABLE II
Refreshening of Bread Stored at 2 and 30°C:
Duncan's Multiple Range Test for Variable Firmness Values[a]

Refreshening Process	Bread: Control Firmness Value	Control Grouping[b]	With Sodium Stearoyl Lactylate Firmness Value	With Sodium Stearoyl Lactylate Grouping[b]	With Atmul 500 Firmness Value	With Atmul 500 Grouping[b]
Bread at 2°C						
Fresh	82	C	96	B	75	B
After first refreshening	99	C	97	B	83	B
After second refreshening	119	B	100	B	74	B
After third refreshening	124	A	120	A	104	A
Bread at 30°C						
Fresh	84	D	82	D	75	D
After first refreshening	146	C	125	C	117	C
After second refreshening	180	B	165	B	140	B
After third refreshening	258	A	198	A	180	A

[a] $\alpha = 0.05$, DF = 32, N = 36. Values are expressed as g·cm and represent an average of nine measurements—three determinations on each of three slices of bread.
[b] Firmness values with the same grouping letter are not significantly different. The grouping letters of different lots of bread are not related to each other.

firming process at higher storage temperatures. The additional components operative at these conditions are protein changes and moisture redistribution, both of which are not heat-reversible (Willhoft 1971, 1973).

Breads with Surfactants. For breads containing SSL stored at 2°C, the firmness values after four days of storage and two refreshenings were similar to those of fresh bread (Tables I and II). Besides forming complexes with gluten (acting as a dough strengthener), this surfactant complexes mainly with the amylose in starch. The complexed polymers resist retrogradation and can be restored to original form. This explains why the original firmness can be more easily regained than in breads without the surfactant. The third refreshening, however, after six days of storage, did not result in complete firmness reversion, probably because of loss and redistribution of moisture, and changes in proteins.

Atmul 500 showed effects similar to those of SSL on bread refreshening. Breads stored at 2°C for four days and subjected to second refreshening could regain a firmness value similar to that found in the fresh bread. Third refreshening yielded incomplete restoration of firmness. Storage at 30°C and refreshening produced better restoration of original firmness by refreshening than in breads without surfactants. However, the effect was less complete than in the breads kept at 2°C, indicating that even in the presence of surfactant factors other than starch retrogradation contributed to firmness.

Moisture Changes During Storage. Moisture losses during storage and refreshening were followed by determinations of the total weight losses of the loaves wrapped in aluminum foil. Minimal weight changes were observed for breads kept at 2°C; breads stored at 30°C lost approximately 1% during storage and treatments. These changes in overall moisture, by themselves, would be of insufficient magnitude to explain firmness differences among breads kept at 2 and 30°C and refreshened. As mentioned previously (Pisesookbunterng 1983), moisture migration from crumb to crust proceeded at a higher rate at 30°C than at 2°C. This condition also contributed to the incomplete reversibility of softness of breads kept at 30°C.

Soluble Starch, Amylose, and Amylopectin Content

Breads Without Surfactants. Table III shows the content and composition of soluble starch in the control bread crumb at varied storage intervals. The soluble starch content of the control bread stored at 2°C for two days and after the first refreshening suggests that the retrograded starch could be reverted to the fresh state. The amount of heat-reversible starch decreased after four days of storage and the second refreshening, and after six days of storage and the third refreshening. The results obtained for soluble starch in the control bread (Table III) agree with the firmness values (Table I).

The soluble starch content obtained for the control bread crumb stored for two days at 30°C and then refreshened indicates that this bread could not be reverted completely to the fresh state. Heat-irreversible protein denaturation and moisture redistribution play a role in bread staling at higher storage temperatures (Kim and D'Appolonia 1977). However, after the second and third refreshenings, the soluble starch content of the control bread stored at 30°C increased to the original value. The reason for these results is not clear, but the increase might be due to additional solubilization of the starch granules as a result of heat treatment.

Breads with Surfactants. The amount of soluble starch extracted from the bread crumb containing SSL and Atmul 500 (Table III) after two days storage at 2°C and the first refreshening indicates that the retrograded starch can easily revert to the original state found in the fresh bread crumb. The soluble starch content in the bread containing SSL and stored at 2°C after the second refreshening, however, did not support the firmness value found for

TABLE III
Refreshening of Control Bread, Bread Containing SSL,[a] and Atmul 500 (Content and Composition of Soluble Starch)[b]

Storage Time (Days)	Refreshening Process	Soluble Starch (%) 2°C	Soluble Starch (%) 30°C	Amylose (%) 2°C	Amylose (%) 30°C	Amylopectin (%) 2°C	Amylopectin (%) 30°C
Control							
0[c]	...	2.04	2.06	0.26	0.26	1.78	1.80
2	Before first refreshening	1.49	1.72	0.08	0.12	1.41	1.60
	After first refreshening	1.98	1.74	0.09	0.12	1.79	1.62
4	No refreshening	1.35	1.49	0.07	0.10	1.28	1.39
	Refreshened once/before second refreshening	1.48	1.53	0.06	0.11	1.42	1.42
	After second refreshening	1.80	2.13	0.07	0.16	1.72	1.97
6	No refreshening	1.34	1.48	0.07	0.11	1.28	1.37
	Refreshened twice/before third refreshening	1.40	2.24	0.05	0.14	1.36	2.10
	After third refreshening	1.68	2.13	0.09	0.12	1.58	2.01
0[c]	...	1.79	1.76	0.12	0.12	1.67	1.64
2	Before first refreshening	1.42	1.66	0.05	0.08	1.37	1.58
	After first refreshening	1.74	1.66	0.06	0.09	1.68	1.57
4	No refreshening	1.33	1.49	0.04	0.08	1.29	1.41
	Refreshened once/before second refreshening	1.42	1.50	0.04	0.08	1.38	1.42
	After second refreshening	1.57	1.50	0.08	0.08	1.49	1.42
6	No refreshening	1.25	1.51	0.04	0.08	1.21	1.43
	Refreshened twice/before third refreshening	1.37	1.55	0.06	0.08	1.31	1.47
	After third refreshening	1.60	1.56	0.08	0.08	1.52	1.48
SSL							
Atmul 500							
0[c]	...	1.74	1.88	0.18	0.18	1.56	1.70
2	Before first refreshening	1.44	1.50	0.08	0.09	1.36	1.41
	After first refreshening	1.70	1.66	0.08	0.09	1.62	1.57
4	No refreshening	1.26	1.38	0.08	0.08	1.18	1.30
	Refreshened once/before second refreshening	1.28	1.49	0.06	0.10	1.22	1.39
	After second refreshening	1.68	1.54	0.08	0.12	1.60	1.42
6	No refreshening	1.20	1.38	0.05	0.09	1.15	1.29
	Refreshened twice/before third refreshening	1.21	1.33	0.06	0.08	1.15	1.25
	After third refreshening	1.72	1.93	0.07	0.12	1.65	1.81

[a]SSL = Sodium stearoyl lactylate.
[b]Values reported are an average of two determinations.
[c]Zero day storage represents bread two hr after removal from oven.

the corresponding bread crumb shown in Table I. The firmness values suggest an essentially complete reversion of the retrograded starch after the second freshening.

The lower level of solubles in bread with surfactants compared to those prepared without them is probably due to the formation of insoluble amylose-surfactant complexes. Also, the decrease of solubles during repeated refreshening may be explained on the same basis: additional solubles formed become bound in the surfactant complex, which reduced the level of solubles.

Similar results were obtained with the bread containing Atmul 500 and stored at 2°C and after the second refreshening. The increased soluble starch content obtained after the third refreshening, however, did not support the firmness value of the corresponding bread stored at the same temperature. These slight increases may be attributed to additional solubilization of the starch granules by application of heat to refreshen the breads.

Table III shows that it was not possible to completely revert the retrograded starch in the bread crumb containing SSL or Atmul 500 stored at 30°C after any of the refreshenings. However, there was a definite increase in the soluble starch content in the bread crumb containing Atmul 500 after the third refreshening. The reason for this result is indicated above.

The soluble starch content data were not entirely correlated with the crumb firmness values. A possible reason may be that firmness values are affected not only by starch but by other components as well. The composition of the soluble starch extracted from all bread crumbs indicates that only the amylopectin was affected by refreshening and that the amount of amylose did not increase upon heating, confirming the study by Schoch and French (1947).

TABLE IV
Triangle Test of Fresh, Refreshened Two Day Stale, and Two Day Stale Bread[a]

Description	Significance at 2°C	Significance at 30°C
Refreshened two day stale bread vs two day stale bread	8**[b]	2
Fresh bread vs two day stale bread	10***[b]	9***[b]
Refreshened two day stale bread vs fresh bread	7*[b]	9***[b]

[a] Results are based on duplicate determinations. Values reported are number of panelists from a 10-member taste panel who were able to discriminate among the bread.

[b] Significant levels are based on Manual on Sensory Testing Method: Sensory Evaluation of Material and Products ASTM Committee E-18, 1968. *** = Significant at the 0.1% level; ** = significant at the 1% level; and * = significant at the 5% level.

Sensory Evaluation

Table IV shows the statistical treatment of the triangle evaluation of fresh bread, refreshened two-day stale bread, and two-day stale bread stored at 2 and 30°C. The taste panel results of

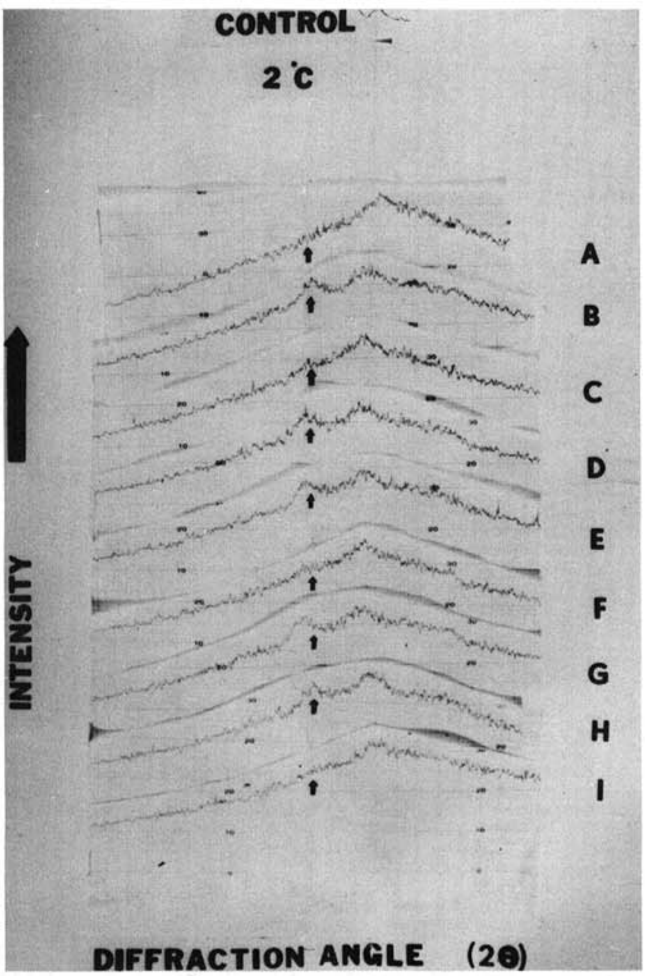

Fig. 1. X-ray diffraction patterns of bread crumb stored at 2°C. A, fresh bread crumb; B, two day storage before the first refreshening; C, two day storage after the first refreshening; D, four day storage without any refreshening; E, four day storage before the second refreshening; F, four day storage after the second refreshening; G, six day storage without any refreshening; H, six day storage before the third refreshening; I, six day storage after the third refreshening.

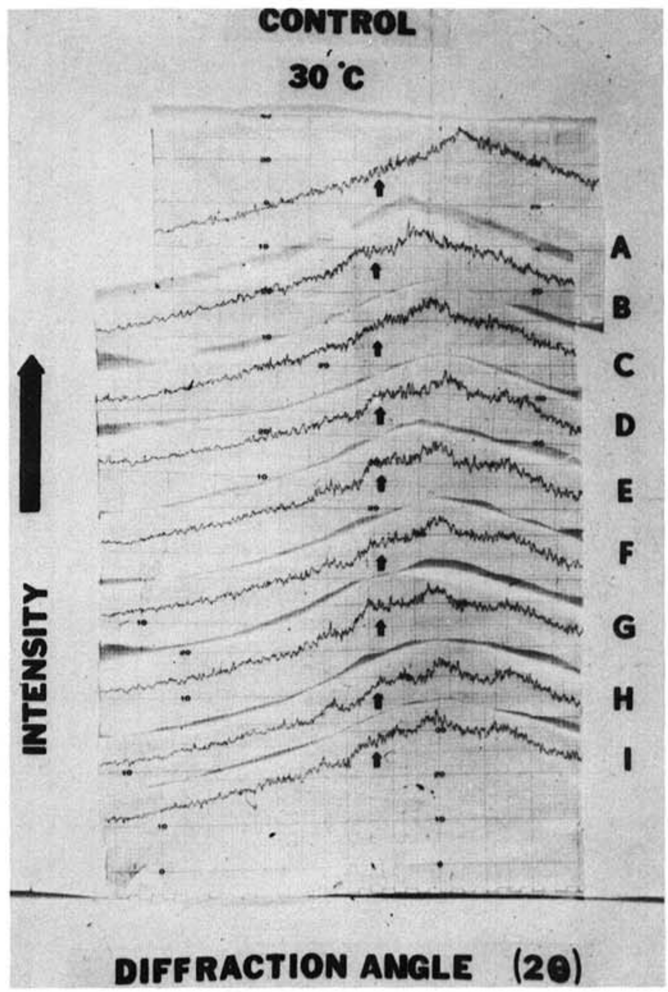

Fig. 2. X-ray diffraction patterns of bread crumb stored at 30°C. A, fresh bread crumb; B, two day storage before the first refreshening; C, two day storage after the first refreshening; D, four day storage without any refreshening; E, four day storage before the second refreshening; F, four day storage after the second refreshening; G, six day storage without any refreshening; H, six day storage before the third refreshening; and I, six day storage after the third refreshening.

the testing of the refreshened two-day stale bread vs two-day stale bread stored at 2°C were significant at the 1% level, whereas the results of the testing of the refreshened two-day stale bread vs fresh bread were significant at the 5% level. This suggests that two-day stale bread stored at 2°C can be refreshened so that it is similar to fresh bread.

Based on crust characteristics, most of the panelists could differentiate between the refreshened two-day stale bread after storage at 2°C and the fresh bread. The crust of the refreshened bread slice after storage at 2°C was somewhat leathery and slightly tough, similar to that of the two-day stale bread slice, whereas the crust of the fresh bread slice was crisp and brittle. A difference in crumb softness between the refreshened bread after storage at 2°C and fresh bread was difficult to detect.

Fresh and refreshened breads stored at 30°C could be distinguished without difficulty. Most panelists could not detect crumb or crust differences in refreshened two-day stale bread and two-day stale bread stored at 30°C. These results indicate that additional factors such as protein alteration and moisture redistribution, which are not heat-reversible, are involved in addition to starch retrogradation at higher storage temperatures.

Although the flavor and aroma of bread lost during storage can be restored by heat application during refreshening (Schoch 1965), a considerable amount of flavorants are lost. Several panelists commented that the flavor and aroma of the refreshened two-day stale bread after storage at 2°C and refreshening were not similar to those in fresh bread. Most likely, some flavor and aroma compounds might volatize from the crust portion, or oxidative changes of these compounds may occur so that they are not reversible by heat.

X-Ray Diffraction Studies

Figures 1 and 2 illustrate the X-ray diffraction patterns obtained on the bread crumbs used in the refreshening studies at storage temperatures of 2 and 30°C, respectively. The intensity of line 5 in the X-ray diffraction pattern increases with bread storage (Zobel and Senti 1959). This line corresponds to the relatively sharp peak located at $2\theta = 17.2°$ or by the d-spacing at 5.16Å, as indicated by the arrows. The distinctive difference in X-ray patterns among fresh, stale, and refreshened stale bread stored at 2 and 30°C is the presence or absence of the relatively sharp peak located at $2\theta = 17.2°$ (d-spacing at 5.16Å) as indicated by the arrows.

After a 2-hr cooling period, fresh bread crumb (Figs. 1A and 2A) showed a very weak or indistinct peak at $2\theta = 17.2°$. For bread stored at 2°C (Fig. 1), this peak in bread crumb before the first, second, and third refreshenings (B, E, and H) as well as bread crumb without any refreshening after four and six days of storage (D and G), respectively, was more distinct than the peak shown by the corresponding samples stored at 30°C (Fig. 2). These results suggest a higher degree of starch retrogradation in bread crumb at the lower storage temperature.

The peak at $2\theta = 17.2°$ in bread crumb stored at 2°C essentially disappeared after the first, second, and third refreshenings (Fig. 1C, F, and I), indicating that the retrograded starch reverted to the fresh state (Fig. 1A). Less complete restoration of retrograded starch to the fresh state was noted with the corresponding bread crumb stored at 30°C (Fig. 2), as illustrated by the incomplete disappearance of the same peak, which indicates that changes other than starch retrogradation are involved at higher storage temperatures.

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