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AGING AND STORAGE STUDIES IN FLOURS AND AIR-CLASSIFIED FLOUR FRACTIONS¹

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ABSTRACT

Flour-aging phenomena were studied in a soft wheat flour and in its air-classified fractions; in a high-protein fraction individually treated with ammonia and chlorine; in a low-protein fraction individually smooth-rolled, ball-milled, and impact-milled; in impact-milled and impact-heat-treated hard wheat flour, soft wheat flour, and low-protein fraction; and in a low-protein fraction ball-milled individually using stone, steel, and brass balls. Flours of about 9% moisture, stored in closed containers alternating between 24° and 32°C. in 6-hr. cycles, did not change in maltose values during 51 weeks. However, when flours in cotton bags were exposed to 58 and 90% r.h. respectively at corresponding temperatures, maltose value decreased considerably in 30 weeks. An explanation is submitted. Former findings about lipids being responsible for flour-aging phenomena are confirmed. Different means by which the total starch granule surface in the sample may be altered are discussed. Upon exposure to steel and brass surfaces lipid decomposition *in situ* was observed, as measured by low iodine value of the petroleum ether extract. The so-called "autolytic degradation" of flour lipids in storage may be explained with or without the presence of lipolytic enzymes within the flour samples.

In the food industries the word *aging* is used generally to denote changes which occur in a food with the lapse of time. More specifically, in the present study the word *aging* is chosen to cover changes, in commercial but unbleached flours, which occur within a definite time period. At the beginning of this period a flour is selected which has a certain entropy level dependent on its history. This entropy level is changed by the addition and/or deduction of energy from an exogenous energy source by various means such as: exposure to high temperature; increase of moisture content; exposure to alternating temperature; exposure to alternating humidity conditions; exclusion of atmospheric air; exposure to gases other than air; exposure to rapid

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agitation; exposure to size reduction; selection of typical particle-size ranges; and exposure to certain combinations of the above or other procedures.

During the storage period the flour undergoes chemical and physical changes which are recorded by changes in test indices used to characterize the *flour-aging* phenomena.

Past investigators found in storage studies that with increasing time gradual hydrolysis of fats occurs, yielding increasing amounts of *fatty acids and/or titratable acidity* (1-6). The intensity of the decomposition is enhanced by higher moisture level in the sample (1,3,5,6) and by a higher temperature level of the storage (1,3,6,7). It appeared that at low moisture level *oxidative rancidity* became enhanced (1). Since the hydrogen-ion concentration was found to be in direct relation with titratable acidity measurements (1,6,8), pH data were recorded in storage studies. It was found, in accordance with acidity level changes, that the *pH* of wheat flour decreases with age, and this change is accelerated by a high moisture content (4,6,9,10,11). Treatment of flour with *chlorine* causes an increase in H-ion concentration (4,12) which continues with subsequent storage time (9). *Peroxide* and *monocarbonyl* values of flour lipids were low and showed little change during the storage period (1). On the basis of the identical pH level of water and ether extracts of freshly milled wheat flours, Johnson and Green thought that the ether-extractable acids are alone responsible for the change in H-ion concentration during storage (13). Schulerud, using aqueous suspensions and three different extraction methods, concluded that fatty acids alone are responsible for increase in flour acidity with longer storage time (14). Gömöry found that the *odor* of fatless flours, and *taste* of bread made from them, were not changed after 18 months of storage, whereas their unextracted control deteriorated under identical storage conditions, developing products of fat decomposition. On the basis of these tests he suggested that the fats are responsible for the recorded changes (5). Fisher *et al.* recorded *maltose* values of four different flours stored in closed containers; they could not observe a progressive change in maltose figures (4). Halton and Fisher recorded decreased pressure of oxygen with increased storage over flours kept in closed containers (10). They found that "when flour is stored in air, as in ordinary commercial practice, the oxidation of flour fat does not apparently occur; although the fat content diminishes, the *iodine value* of the fat is unaffected during many months of storage." This observation coincides with those of Kozmin (6) and Dörner (2), who could not detect significant changes in iodine value of the petroleum ether extracts of flour samples stored

for a limited time. Gömöry (5) recorded a decrease in iodine value from 116.16 to 100.20 during 15 months of storage in the fat extracted from a flour having about 14% moisture content. During the same storage period the iodine value of flour fat decreased from 111.10 to 78.50 if the flour was dried, previous to storage, to about 8% moisture content. Smith and Andrews recorded that the *oxygen uptake* of flours mixed into a dough in oxygen atmosphere did not change if the flour was stored at -20°F ., but increased if the flour was stored at room storage conditions and the increase was amplified with the presence of moisture (15) in the stored flour. Cosgrove measured, with longer storage time, increased amount of oxygen uptake by flours milled from Manitoba and local English wheat using a buffer suspension of 6.5 pH at 25°C . temperature (16).

From the above review it appears that investigators restricted themselves mostly to practical storage studies which aimed to find indices for deteriorated flours unfit for consumption. Such studies simulated natural conditions and used only a limited amount of exogenous energy to accelerate the changes of aging in flours. In the present study a relatively large amount of exogenous energy is applied, and it is hoped that by such amplification more light can be shed upon this very complex phenomenon.

Materials and Methods

The materials used in the different phases of these studies are described in Table I, which includes convenient reference to the data of pertinent tests using the lower-case letters in parentheses. The letters also mark the individual flours selected for observation having the initial entropy level.

The analytical indices were determined by methods described in *Cereal Laboratory Methods* and identified by their test numbers: protein 67.1, moisture 48.3, ash 9.1, maltose (diastatic activity) 24.1, and pH 60.2 (17). The standard error of the maltose determination is estimated to be $\pm 2-3$ mg./10/g. flour (18).

The fat content was determined by the use of petroleum ether in a Soxhlet extractor and by acid hydrolysis following AOAC procedures 22.028 and 13.119 respectively (19). The fatty acid content was determined by AOCS method CA 5a-40 (20). For the determination of iodine value about 0.25 g. petroleum ether extract was used. This was obtained by AOAC method 22.028 (19), except that the last 25 cc. of extractant was evaporated under vacuum using nitrogen. The determination of iodine value used AACC method 41.2 (17), modified in that the procedure included manipulations by the use of

TABLE I
ANALYTICAL DATA IDENTIFYING AND CHARACTERIZING PARENT FLOUR SAMPLES USED IN
THE DIFFERENT PHASES OF THE STUDIES

FLOUR OR FRACTION	YIELD	PROTEIN	ASH	PARTICLE SIZE	MASS
				RANGE EXPRESSED IN CRITICAL CUTS OF THE OPERATION	MEDIAN SIZE
	%	%	%	SED μ	SED μ
(a) Soft red winter wheat (parent) flour	100	8.2	0.319	min.-max.	30.5
(b) High-protein fraction of (a)	5.8	20.2	0.387	0-11.7	4.6
(c) Low-protein fraction of (a)	32.8	3.0	0.291	21.2-32.0	25.7
(d) Fraction of smaller and larger endosperm chunks of (a)	41.5	8.1	0.324	32.0-max.	41.5
(e) Fraction of larger endosperm chunks of (a)	27.4	9.0	0.332	40.0-max.	46.8
(f) Low-protein fraction of a soft red winter wheat (parent) flour	32.3	3.1	0.293	21.6-30.5	27.0
(g) High-protein fraction of a soft red winter wheat (parent) flour	6.1	20.7	0.427	0-10.8	4.3
(h) Hard red spring wheat flour	100	12.3	0.375	min.-max.	61.0
(i) Intermountain soft wheat flour	100	9.2	0.342	min.-max.	24.8
(j) Low-protein fraction of short patent soft red winter wheat flour	...	3.3	23.5
(k) Low-protein fraction of short patent soft red winter wheat flour	40.6	3.6	0.280	20.5-32.0	23.8
(l) Ohio soft red winter wheat short patent flour	100	7.5	0.318	min.-max.	27.0
(m) Kansas hard red winter wheat flour	100	11.1	0.420	min.-max.	55.0

nitrogen to prevent direct contact of the extract with air, and in that chloroform was used instead of CCl_4 to suspend the extract.

The alkaline water retention capacity (AWRC) was obtained by the method of Yamazaki. Its standard error is estimated to be $\pm 1.41\%$ (21). The average particle size was determined with the Fisher apparatus, using its operational manual (22,23,24). The standard error using the same instrument (as in the present case) was estimated by Croteau (25) to be $\pm 4\%$ of the figure. Figures used in this study are averages of two runs reducing the error limit to $\pm 2.8\%$. The mass median size was obtained from the size distribution data determined by the Whitby sedimentation method (26), using the time schedule and determination as discussed by Gracza (24).

The critical cuts of air-classification steps were determined by the use of the particle-size distribution and the maximum ordinate differ-

ence (sharpness) method (27). Yield figures of fractions are expressed in percent of their respective parent flour (28). The analytical data are expressed on 14% moisture basis. The oxygen, ammonia, and chlorine used in the test were commercial grade gases.

Tests and Results

A. Exposure of Flour and Its Typical Air-Classified Fractions to Abusive Storage

Wheat flour and its typical air-classified fractions (28), namely *a*, *b*, *c*, *d*, and *e* flours of Table I with moisture content natural to their processing conditions, were subjected to an accelerated abusive storage simulating adverse atmospheric conditions in certain geographic areas.

TABLE II
ANALYTICAL DATA OF AN UNBLEACHED, UNTREATED OHIO SOFT RED WINTER WHEAT FLOUR AND THOSE OF ITS FOUR AIR-CLASSIFIED FRACTIONS STORED IN ABUSIVE STORAGE^a

	MOISTURE ^b	MALTOSE	AWRC	AVERAGE PARTICLE SIZE BY PERMEABILITY (FISHER)	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	pH
	%	mg./100 g.	%	μ	%	%	
(a) Soft wheat parent flour							
1)	12.3	107	54.9	11.6	0.91	11.8	5.63
2)	13.0	85	51.7	12.6	0.83	22.9	5.09
3)	12.9	80	52.5	12.3	0.90	28.1	4.64
(b) High-protein fraction; (protein fragments, smaller starch granules)							
1)	7.2	171	104.3	3.7	1.78	14.0	5.50
2)	12.3	131	87.0	4.0	1.89	32.0	4.95
3)	12.0	115	93.2	3.9	1.83	42.8	4.55
(c) Low-protein fraction; (larger starch granules)							
1)	8.5	98	59.5	15.8	0.49	12.4	5.82
2)	10.0	82	58.1	17.8	0.40	21.7	5.31
3)	13.0	67	60.5	18.3	0.40	29.9	4.85
(d) Fraction of medium and large endosperm fragments							
1)	10.4	83	51.6	16.2	0.88	11.8	5.62
2)	12.9	65	49.1	18.3	0.93	15.4	5.08
3)	12.9	61	51.3	19.4	0.90	19.4	4.60
(e) Fraction of larger endosperm fragments							
1)	9.8	77	54.7	20.2	1.04	10.8	5.81
2)	13.0	63	53.1	22.6	1.02	14.8	5.11
3)	12.9	61	54.1	23.1	1.03	19.1	4.6

^a Samples stored in 5-lb. cotton bags in an accelerated-storage room, where the "climatic" condition alternated in 6-hr. cycles between 58% r.h. at 24°C. and 90% r.h. at 32°C. The lines numbered 1, 2, and 3 in each group indicate data obtained within 10 days of (respectively): Feb. 10, 1957, on samples without any storage; June 17, 1957 (i.e., after 17 weeks of accelerated storage); Sept. 17, 1957 (i.e., after 30 weeks of accelerated storage).

^b Initial moisture levels of the samples are natural to flour-processing conditions.

The individual flour samples were exposed in 5-lb. cotton bags in absence of light (except when handled) to a climatic condition which alternated in 6-hr. cycles between 58% relative humidity (r.h.) at 24°C. and 90% r.h. at 32°C. The bags were flattened and placed individually so the moisture of air had ready access to the flour. At the beginning and at the end of 17 and 30 weeks of accelerated storage, estimated to be equivalent to 68 and 120 weeks of storage respectively in the simulated geographical locations, samples were withdrawn and analyzed within 10 days of withdrawal. The results are shown in Table II.

TABLE III
ANALYTICAL DATA OF AN UNBLEACHED, UNTREATED OHIO SOFT RED WINTER WHEAT FLOUR AND THOSE OF ITS FOUR AIR-CLASSIFIED FRACTIONS IN ACCELERATED STORAGE^a

	MOISTURE ^b	MALTOSE	AWRC	AVERAGE PARTICLE SIZE BY PERMEABILITY (FISHER)	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	pH
	%	mg./100 g.	%	μ	%	%	
(a) Soft wheat parent flour							
1)	14.5	110	53.1	11.8	0.92	13.8	5.74
2)	13.4	82	52.4	12.5	0.88	22.1	5.20
3)	13.0	78	52.5	12.6	0.86	28.4	4.62
(b) High-protein fraction (protein fragments and smaller starch granules)							
1)	14.4	159	97.8	4.0	1.79	15.1	5.57
2)	12.6	132	88.9	4.0	1.85	32.2	4.90
3)	12.1	115	89.2	4.1	1.80	42.3	4.48
(c) Low-protein fraction (larger starch granules)							
1)	13.9	92	57.5	14.8	0.42	13.7	5.86
2)	13.4	76	58.7	14.6	0.46	19.9	5.23
3)	13.3	67	59.9	15.0	0.45	28.3	4.72
(d) Fraction of medium and larger endosperm fragments							
1)	14.7	84	49.4	17.3	0.87	11.6	5.75
2)	13.3	67	49.2	19.1	0.87	17.4	5.17
3)	13.2	61	52.1	19.1	0.91	22.0	4.59
(e) Fraction of larger endosperm fragments							
1)	14.5	75	53.7	20.0	0.98	10.7	5.73
2)	13.3	61	52.3	22.4	1.00	14.8	5.14
3)	13.0	61	54.1	22.6	1.01	21.8	4.61

^a Samples stored in 5-lb. cotton bags in an accelerated storage room where the climatic condition alternated in 6-hr. cycles between 58% r.h. at 24°C. and 90% r.h. at 32°C.

Lines 1, 2, and 3 in each group: see footnote a in Table II.

^b The initial moisture level of the samples was raised to about 14.5% in a humidity cabinet before exposure to abusive storage.

Table III shows analytical data of the same flours and of the same exposure except that the samples were brought to about 14.5% mois-

ture level in a conditioning cabinet before they were placed into the accelerated storage room.

On the basis of the data of Morey *et al.* (29), it is estimated that the moisture content of flour samples alternated in the storage between $\pm 1-1.5\%$ around their respective averages. In the drier samples the moisture increased, and in the moister samples the moisture decreased, approximating their respective equilibrium moisture. The data give some indication that the rate of reaching this equilibrium is lower with lower protein content. The maltose values decreased with increasing exposure during the abusive accelerated storage. The percentual maltose drop within 30 weeks indicates that the rate of change is not constant but appears to vary with the initial moisture level, specific surface (inversely proportional to the Fisher value), and starch content, or rather, condition of starch, in the flour samples. This observation suggests the involvement of specific surface, moisture, and specific starch properties into the phenomena that cause the decreasing maltose values during the applied abusive accelerated storage.

The average particle size as determined by the permeability of air through a porous bed of flour samples (Fisher) increased slightly with the duration of abusive storage, indicating some agglomeration by loss of surface. The rate of this surface loss (increase of Fisher value) is slightly greater in the series with natural moisture level as compared to the series with raised moisture level, involving the moisture content of samples into the phenomena observed.

While the fatty acid content of the petroleum ether-extractables increased with storage time, the ether-extractables did not show any observable trend. The pH data, while slightly differing in the individual flour fractions, decreased with the abusive storage as expected.

B. Exposure to Different Kinds of Size Reduction and Subsequent Abusive Storage

A low-protein fraction of a soft wheat flour sample (28), identified in Table I with *f*, was subjected individually to roll-, ball-, and multiple-impact grinding. The first batch of the sample was passed four times between the closely set smooth rolls of an Allis experimental mill. The second batch of the same sample was ball-milled at 72 r.p.m. for 48 hr. in 1-gal. jars containing stone balls. The third batch of the sample was impact-milled in a commercial grinder.

The individually ground flour samples and their parent flour were exposed in 5-lb. cotton bags to the same abusive storage as samples *a*, *b*, *c*, *d*, and *f* in the accelerated storage chamber. At the end of 14

TABLE IV
ANALYTICAL DATA OF LOW-PROTEIN FRACTION OF AN UNBLEACHED, UNTREATED OHIO
SOFT RED WINTER WHEAT FLOUR AND THOSE OF ITS ROLL-, BALL- AND
IMPACT-GROUND DERIVATIVES IN ABUSIVE STORAGE^a

	MOISTURE	MALTOSE	AWRC	MASS MEDIAN SIZE	AVERAGE PARTICLE SIZE BY PERME- ABILITY (FISHER)	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	pH
	%	mg./100 g.	%	SED μ	μ	%	%	
(f) Low-protein fraction control								
5) ^b	9.8	97	58.8	26.4	14.0	0.42	15.2	...
6)	13.3	69	61.5	24.8	15.7	0.38	20.9	5.03
7)	12.3	62	60.3	23.6	16.4	0.46	24.8	4.55
Low-protein fraction passed four times between polished smooth rolls								
5)	7.6	396	89.3	24.8	12.4	0.42	15.1	...
6)	13.0	243	92.1	25.6	13.6	0.41	19.8	5.01
7)	12.1	163	92.5	24.0	13.7	0.44	26.1	4.60
Low-protein fraction ball-milled for 48 hr.								
5)	9.3	569+	215.3	24.6	8.1	0.39	18.2	...
6)	12.9	248	230+	22.8	10.5	0.35	22.0	5.07
7)	12.0	111	443.1	25.2	14.5	0.39	26.4	4.67
Low-protein fraction after multiple impact handling in air suspension								
5)	5.6	122	62.1	23.0	12.2	0.41	15.1	...
6)	12.8	85	62.9	21.8	14.0	0.49	19.6	5.06
7)	12.1	70	64.1	22.8	14.5	0.42	29.6	4.65

^a Samples stored in 5-lb. cotton bags in an accelerated storage room where climatic conditions alternated between 58% r.h. at 24°C. and 90% r.h. at 32°C.

^b Lines numbered 5, 6, and 7 in each group indicate data obtained within 10 days of (respectively): April 10, 1957 (without any storage); July 15, 1957 (after about 14 weeks of accelerated storage); Sept. 17, 1957 (after about 23 weeks of accelerated storage).

and 23 weeks of storage, parts of the samples were withdrawn and analyzed within 10 days of the withdrawal. The results are shown in Table IV. The particle-size data, as measured by the sedimentation method and permeability method (Fisher), indicated only a small degree of size reduction except for the ball-milled sample as measured by the Fisher value. The particle size increased during abusive storage. The percentage of increase was 17.1, 10.5, 79.1, and 19.0% in the parent and the roll-, ball-, and impact-milled samples respectively during the 23-week storage period. The maltose values increased as a result of the size reduction procedures. The increase was slight in the impact grinder, more in the smooth-rolled stock, and largest in the ball-mill under the chosen experimental conditions. The maltose value decreased with increasing exposure in abusive storage. The percentage of maltose drop in storage indicates that the change in entropy is not proportional to the value at the initial condition of the sample in the differently ground series. The percentage of maltose

decrease in the 23-week storage period is 36.0, 58.8, 80.4, and 42.6% in the parent, roll-milled, ball-milled, and impact-handled samples respectively. The water-retention capacity of the samples increased 2.5, 3.5, 51.5, and 3.1% in the parent, roll-milled, ball-milled, and impact-milled series respectively in the 23-week storage period. The amount of petroleum ether-extractables changed very slightly. Free fatty acid content of the samples increased with attendant decrease in pH. The rates of decrease in fatty acid content during the 23-week period were 38.7, 34.5, 31.4, and 49.0% in the parent, roll-milled, ball-milled, and impact-milled samples respectively.

C. Exposure to Ammonia and Chlorine and Subsequent Abusive Storage

A high-protein fraction of a soft wheat flour (28) described in Table I (see sample *g*) was treated with ammonia to raise its pH from 5.70 to 6.85; another batch of the same sample was treated with chlorine to drop its pH to 4.22. The gas was forced into a 1-cu.-ft. wooden tumbling box in which the sample was thoroughly mixed with the gas. The individual samples including their parent flour were subsequently exposed in 5-lb. cotton bags to the same abusive storage as samples *a*, *b*, *c*, *d*, and *f*, in the accelerated storage chamber. At the

TABLE V
ANALYTICAL DATA OF A HIGH-PROTEIN FRACTION OF AN UNBLEACHED, UNTREATED OHIO SOFT RED WINTER WHEAT FLOUR AND THOSE OF ITS AMMONIA- AND CHLORINE-HANDLED VARIABLES IN ABUSIVE STORAGE^a

	MOISTURE	MALTOSE	AWRC	AVERAGE PARTICLE SIZE BY PERMEABILITY (FISHER)	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	pH
	%	mg./100 g.	%	μ	%	%	
(g) High-protein fraction control							
5) ^b	8.5	150	95.9	3.6	1.68	19.7	5.70
6)	12.6	144	87.1	3.8	2.05	33.9	5.02
7)	12.4	114	89.3	4.0	2.05	44.0	4.65
High-protein fraction treated with ammonia in bleaching box							
5)	7.6	147	99.1	3.8	1.78	17.7	6.85
6)	12.6	132	85.6	3.9	2.04	34.6	5.08
7)	12.3	102	86.9	4.2	2.00	42.3	4.56
High-protein fraction treated with chlorine in bleaching box							
5)	7.8	154	93.4	3.7	1.61	14.8	4.22
6)	12.3	140	83.9	3.9	1.82	17.3	4.11
7)	12.3	119	88.9	4.1	2.14	17.1	3.72

^aSamples stored in 5-lb. cotton bags in an accelerated storage room where climatic conditions alternated between 58% r.h. at 24°C. and 90% r.h. at 32°C.

^bLines numbered 5, 6, and 7 in each group: see footnote b, Table IV.

end of 14 and 23 weeks, portions of the samples were withdrawn and analyzed within 10 days of withdrawal. The results are shown in Table V. The maltose value decreased with increased abusive exposure. In the 23 weeks of storage 24.0, 44.0, and 22.7% decrease of maltose figures were recorded respectively in the parent, ammonia-treated, and chlorine-treated samples. The average particle size by Fisher increased about 10% in the same time span, indicating agglomeration by surface loss. The petroleum ether-extractables increased together with the fatty acid content during storage. The rate of fatty acid increase in the 23-week period was 123, 139, and 15.5% in the parent, ammonia-treated, and chlorine-treated samples respectively.

D. Exposure to Multiple Impact in Air Suspension at 57°C. and 117°C. and Subsequent Storage in Closed Containers

A hard spring wheat flour, an Intermountain soft wheat flour, and a low-protein fraction of a soft wheat flour, identified as *h*, *i*, and *j*

TABLE VI
ANALYTICAL DATA OF AN UNBLEACHED, UNTREATED HRS WHEAT FLOUR AND ITS
VARIABLES HANDLED IN AIR SUSPENSION AT 57° AND 117°C. AND STORED IN
CLOSED MASON JARS

(In storage, temperature alternated between 24° and 32°C. in 6-hr. periods)

STORAGE	MOISTURE	MALTOSE	AWRC	MASS MEDIAN SIZE	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	IODINE VALUE OF PETROL ETHER EXTRACT
<i>weeks</i>	%	mg./10 g.	%	SED μ	%	%	
(h) Hard spring wheat flour parent							
0	9.5	209	68.6	61.0	0.71	14.3	110.6
4	9.8	197	69.8	..	0.69	17.0	111.3
15	9.1	210	68.4	..	0.76	17.6	109.6
32	9.6	...	68.4	..	0.70	23.3	108.5
51	9.2	198	66.7	..	0.68	24.8	108.4 ^a
76	0.70	29.8	105.0
Parent size reduced by multiple impact in air suspension at 57°C.							
0	5.8	272	73.0	26.2	0.64	13.6	102.4
4	6.2	244	74.8	..	0.63	15.6	107.2
15	6.2	264	73.9	..	0.72	16.1	105.9
32	6.3	...	73.2	..	0.63	18.2	103.5
51	6.4	263	74.2	..	0.63	19.0	102.4 ^a
76
Parent size reduced by multiple impact in air suspension at 117°C.							
0	2.2	280	78.8	27.2	0.57	7.8	105.1
4	2.4	277	79.3	..	0.58	8.8	108.8
15	2.5	277	87.8	..	0.65 ^p	7.6	107.8
32	2.8	...	82.1	..	0.49	11.0	90.0
51	3.3	274	81.7	..	0.22	18.3	61.4 ^a
76

^a Result of a single run; all other iodine values are averages of two runs.

TABLE VII
ANALYTICAL DATA OF AN UNBLEACHED, UNTREATED INTERMOUNTAIN SOFT WHEAT FLOUR
AND ITS VARIABLES HANDLED IN AIR SUSPENSION AT 57° AND 117°C. AND
STORED IN CLOSED MASON JARS^a

TIME IN STORAGE	MOISTURE	MALTOSE	AWRC	MASS MEDIAN SIZE	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	IODINE VALUE OF PETROL ETHER EXTRACT
<i>weeks</i>	%	mg./10 g.	%	SED μ	%	%	
(i) Intermountain soft wheat flour parent							
0	8.3	92	56.3	24.8	0.70	26.4	110.3
4	9.1	95	58.0	..	.69	27.8	113.0
15	9.0	97	50.9	..	.74	37.7	114.6
32	9.3	..	57.8	..	.68	40.9	110.0
51	9.4	95	57.3	..	.66	45.0	109.4 ^b
76	9.4	0.68	49.2	107.6 ^b
Parent size reduced by multiple impact in air suspension at 57°C.							
0	5.6	110	62.6	21.0	0.47	21.9	106.4
4	6.0	100	64.1	..	.56	27.6	115.0
15	5.9	102	61.6	..	.57	30.9	108.0
32	5.8	..	65.7	..	.60	29.2	102.8
51	5.6	102	67.1	..	.55	28.2	91.0 ^b
76	7.1	0.49	33.5	78.7
Parent size reduced by multiple impact in air suspension at 117°C.							
0	67.7	18.2	0.52	14.0	108.8
4	2.3	90	70.1	..	.50	16.2	108.4
15	2.2	91	73.5	..	.36	18.4	77.7
32	2.3	..	71.0	..	.24	24.3	64.7
51	2.8	92	58.9	..	.20	25.6	61.7 ^b
76	4.7?	0.20	24.3	61.0 ^b

^a In storage, temperature alternated between 24° and 32°C. in 6-hr. periods.

^b Result of a single run; all other iodine values are averages of two runs.

samples respectively in Table I, were selected. Individual batches of the three samples were impact-milled at 57°C. and 117°C. as measured at the mill exit in the discharge air. The ground samples and their parent stock were stored in individual closed Mason jars at their natural moisture levels obtained in the handling procedure, and were exposed to heating and cooling cycles alternating in 6-hr. periods between 24° and 32°C. (76° and 90°F.). Portions of the samples were withdrawn after 4, 15, 32, 51, and 76³ weeks of storage and analyzed within 10 days of the withdrawal, as shown in Tables VI, VII, and VIII. The maltose values changed very slightly with storage time, within the error limit of the determination. The petroleum ether-extractable fats decreased slightly when the sample was impact-ground, and slightly more if the impact-grinding was performed at elevated temperature. The storage in closed containers at relatively low moisture level did not change the amount of ether-extractables

³ Only moisture and lipid indices were used on these samples.

TABLE VIII

ANALYTICAL DATA OF A LOW-PROTEIN (3.3%) FRACTION OBTAINED FROM AN UNBLEACHED, UNTREATED SOFT RED WINTER WHEAT FLOUR AND ITS VARIABLES HANDLED IN AIR SUSPENSION AT 57° AND 117°C. AND STORED IN CLOSED MASON JARS^a

TIME IN STORAGE	MOISTURE	MALTOSE	AWRC	MASS MEDIAN SIZE	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	IODINE VALUE OF PETROL ETHER EXTRACT
<i>weeks</i>	%	mg./10 g.	%	SED μ	%	%	
(j) Low-protein fraction of a soft red winter wheat flour parent							
0	8.3	68	59.7	23.5	0.26	32.8	109.9
4	8.3	64	60.0	..	0.26	33.2	110.0
15	8.2	66	58.1	..	0.29	34.5	111.4
32	8.5	0.30	41.1	107.5
51	8.3	73	58.4	..	0.25	42.6	106.7 ^b
76	8.4	0.29	41.9	101.5 ^b
Parent size reduced by multiple impact in air suspension at 57°C.							
0	6.0	82	59.6	22.2	0.25	31.6	109.8
4	6.4	63	61.3	..	0.25	32.9	112.4
15	6.2	74	58.5	..	0.26	35.7	109.4
32	6.5	0.21	38.4	107.8
51	6.5	83	54.3	..	0.23	40.4	105.1 ^b
76	7.8 ^p	0.24	36.0	91.4 ^b
Parent size reduced by multiple impact in air suspension at 117°C.							
0	2.2	71	62.2	21.5	0.19	13.1	106.7
4	2.7	76	61.8	..	0.16	15.7	104.5
15	2.5	72	70.5	..	0.12	20.3	69.9
32	2.9	0.07	31.0	63.0 ^b
51	3.0	84	69.6	..	0.10	22.1	56.1 ^b
76	4.2	0.12	15.1	..

^a In storage, the temperature alternated between 24° and 32°C. in 6-hr. periods.

^b Result of a single run; all other iodine values are averages of two runs.

except in the sample that had been impact-ground at 117°C. temperature level, where it decreased to less than half of its original value. The fatty acid content of the fats was slightly lowered by impact-grinding. The higher temperature level enhanced fatty acid loss. Fatty acids increased with storage time; however, the rate of increase appears to be dependent on impact-grinding in air suspension previous to the storage and on the temperature level of the air suspension. The iodine value of the petroleum ether extract shows a slight initial increase during storage, but with longer storage it decreases to almost half of its original level. The history of the sample, the air-handling, and the temperature appear to affect the rate of such change.

E. Exposure to Metallic Surfaces in the Ball Mill

A low-protein fraction of a soft wheat flour (28), identified by the letter *k* in Table I, was ball-milled in individual batches for 1, 6, and

TABLE IX

ANALYTICAL DATA OF A LOW-PROTEIN (3.6%) FRACTION OF AN UNBLEACHED, UNTREATED OHIO SOFT RED WINTER WHEAT FLOUR AND ITS STONE-, STEEL-, AND BRASS-BALL-GROUND VARIABLES^a

	BALL-MILLING TIME	MOISTURE	MALT-OSE	CA-PACITY	SED	AVERAGE PARTICLE SIZE BY PERMEABILITY (FISHER)	FAT BY ACID HYDROLYSIS	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	IODINE VALUE OF PETROL ETHER EXTRACT	pH		
	hours	%	mg./10 g.	%	μ	μ	%	%	%				
(k) Low-protein fraction													
Data obtained within 15 days of milling with indicated balls	Parent	0	8.9	110	58.3	23.8	18.0	0.78	0.33	23.8	99.3	5.72	
	Porcelain balls	1	8.4	107	59.6	..	16.0	0.85	.31	23.2	101.0	5.77	
		6	9.2	140	61.7	..	15.2	0.79	.29	25.7	102.5	5.80	
		24	9.0	182	67.5	21.5	11.2	0.92	.27	27.4	95.5	5.81	
	Steel balls	1	9.0	114	58.5	..	16.1	0.73	.32	24.1	87.3	5.78	
		6	8.9	116	60.1	..	15.1	0.87	.29	26.8	85.2	5.77	
		24	9.0	127	63.0	22.5	15.1	0.82	.27	28.2	67.1	5.77	
	Brass balls	1	8.7	109	58.4	..	16.5	0.78	.31	26.1	68.0	5.72	
		6	8.9	114	62.9	..	15.8	0.80	.21	31.9	48.3	5.68	
		24	8.8	133	65.3	20.5	14.3	0.93	.17	43.3	30.4	5.42	
	Data obtained after 20 weeks of storage in plastic bags at room temperature	Parent	0	9.138	28.2	105.5	..
		Porcelain balls	1	8.732	24.4	103.4
6			8.929	27.3	107.4	..
24			9.027	32.4	110.7	..
Steel balls		1	9.137	23.0	107.1	..
		6	9.028	30.7	107.4	..
		24	8.932	27.5	109.8	..
Brass balls		1	8.814	25.4	48.7	..
		6	9.114	27.3	37.9	..
		24	9.0	0.14	25.5	45.2	..

^a Some lipid indices are shown after 20 weeks of storage in room conditions.

24 hr. using stone, steel, and brass balls in the 1-gal. jars of a laboratory ball-mill. The samples were stored in polyethylene bags at ordinary room conditions for 20 weeks. The results of the analytical tests are presented in Table IX. The average particle size by Fisher and the maltose data indicate in a general fashion the degree of size reduction obtained in the ball-grinding procedure. The petroleum ether-extractables decreased slightly; the fatty acid content increased slightly with increasing ball-milling time. The iodine value of the petroleum ether extract decreased with ball-milling time. With the moisture level available in the samples and with increasing ball-milling time, the gradient of iodine value was small when stone balls were used but increased if metallic surfaces, especially brass, were present. Subsequent storage caused the petroleum ether-extractables of the samples handled with brass balls to decrease. After 20 weeks of storage the petroleum ether-extractables showed an increase in iodine value in the steel-ball-handled stock, but a further decrease in the brass-ball-handled stock.

Discussion

The studies of this paper selected in five series certain initial conditions of flour and/or certain combinations of specific exposures to change the entropy level of flours.

Series A (Tables II and III) used the selection of typical particle size ranges of a soft wheat flour obtained by air classification; exposure to alternating temperature level; exposure to alternating humidity level; and selection of initial moisture level for the samples.

Series B (Table IV) used the selection of the low-protein fraction of a soft red winter wheat flour; exposure to size reduction by smooth rolls, ball mill, and impact grinder; exposure to alternating temperature level; and exposure to alternating humidity level for the samples.

Series C (Table V) used the selection of the high-protein-fraction of a soft wheat flour; selection of tumbling box mixer for the exposure to ammonia or chlorine gases; exposure to alternating temperature level; and exposure to alternating humidity level for the samples.

Series D (Tables VI, VII, and VIII) used the selection of three flours: hard spring wheat, Intermountain soft wheat, and a low-protein fraction of a soft red winter wheat flour; exposure to rapid agitation by air as in an impact mill; selection of the temperature of the air suspending the particles in rapid agitation; exposure to alternating temperature levels; and exclusion of atmospheric air for the samples.

Series E (Table IX) used the selection of the low-protein fraction

of a soft red winter wheat flour; exposure to multiple impact in absence of rapid agitation by air in a ball mill; exposure to stone, steel, and brass (copper, zinc) surfaces by the choice of balls; and exposure to storage in room condition excluding atmospheric air for the samples.

The following evaluation of flour aging phenomena rests on the results of the tests described in these studies and upon results recorded by workers in the literature. The evaluation follows the changes in *flour indices* (maltose, pH, fat, fatty acids, iodine value, AWRC) recorded and deemed to be meaningful in characterizing the changed condition of flours upon the specific combination of exposures in the test systems.

Variation of Maltose in the Five Test Systems and Its Analysis.

The relatively mild storage condition of alternating temperature between 24° and 32°C. in closed jars did not modify the condition of flour to change the maltose value in any considerable degree (see Series D). However, if the flours were exposed to the same temperature alternation but with access of the humidity in the air, they underwent changes causing considerable decrease in their maltose value practically independent from their initial moisture content (see Series A, B, and C). This change appears to be independent of lipid indices (see fat, fatty acid, pH, and iodine value indices in Tables V, VI, VII, and VIII) within the tested ranges; however, it appears to depend on the amount or rather on the condition of the starch granules and on the specific surface available for moisture deposition or moisture loss. If the initial *moisture level* was relatively high (in the 14.5% range), as in the samples of Table III, the percentages of maltose decrease in the 30-week period were 29.0, 27.7, 27.7, and 18.7% respectively in the parent and the high-protein, low-protein, and chunk fractions, whereas in the same samples at lower moisture level (Table II) the percentages of decrease are 25.2, 32.8, 21.4, and 20.8% respectively. If the starch granules are cleaved (damaged) in different degree, as in the low-protein fraction (Table IV) consisting mainly of major starch granules and thus more prone for cleavage in different degree by the use of different equipment, the gradient of changes contributing to the decrease of maltose value became smaller with less *cleaved starch granule* in the initial sample: it was 36.0, 58.8, 80.4, and 42.6% in the parent, roll-milled, ball-milled, and impact-milled series respectively during the 23 weeks of exposure. In samples of relatively *low specific surface* (high Fisher), i.e., in the chunk fraction, the gradient of maltose figure is relatively low.

Analysis. Accepting the explanations of Alsberg, Sandstedt, Dracon,

and others, that the autolytic saccharogenesis in a flour is dependent upon the availability of amylolytic enzymes, substrates, and water, or more specifically upon the behavior of starch granules at their cleaved surfaces in the presence of excessive water (30,31,32,33,34,35), and considering that in the closed container-temperature alternating series (D) of the present tests the maltose value did not change, i.e. enzymes and substrates were available in the same degree during the storage period, it is suggested that in the abusive storage (A, B, and C series) the starch granules underwent some changes which rendered the substrate less available with increasing abusive storage.⁴ Since the moisture content of the air, commonly called humidity, occurs in smaller and larger droplets (aggregates of H₂O molecules) suspended in the air,⁵ in abusive storage local hydration and dehydration can occur on the surface of the particles in a frequency determined by the 6-hr. alternating cycle and total storage period. The moisture droplets of smaller size produced by the nozzle of the humidifier are carried by the circulating air through the cotton bags into the channels created by the void space within the bulk of the flour. The volume of these channels is relatively large; about 72.1 and 61.5% of the total bulk volume in a soft and in a hard wheat flour as measured by bulk density data and expressed as porosity (24,28). Depending upon 1) the size of the channels, 2) the size of the droplets (aggregates of more than one water molecule), 3) the moisture level of the particles available at their surface region, 4) the chemical affinity for water of the compounds available at the particle surfaces, and 5) the speed of air travel through the channels, more or less water is deposited to and evaporated from the surface regions of the particles. Since the uncleaved (undamaged) surfaces of the starch granules are much less prone to absorb water as compared to the cleaved (damaged) surfaces (33), one would expect relatively large changes on the cleaved surfaces during the storage time. The rapid hydration (high chemical affinity for water) of the starch molecules at the cleaved surfaces, as can be observed microscopically (33), would also direct attention to the regions of internal

⁴The degree of dependence of the maltose figure on the substrate condition and/or upon the availability of amylases obviously cannot be resolved until either or both of these factors are attempted to be characterized with the amount of sugars produced by amylolytic scission, usually through manipulations in the testing procedures. For an unequivocal, academic answer to this problem the two factors, i.e., substrate limitation and amount of enzymes, need to be measured independently. In the lack of such measurements today, one is forced to resort to the adopted but arbitrary restrictions in the maltose determination procedure and to the best possible estimation of limiting conditions affecting the two factors. In the present studies the availability and/or amount of enzymes during the storage are estimated to be unchanged, since at the used temperature and moisture levels their "denaturation" could be only negligible.

Mold and bacteria in flour may use some sugars in their metabolism; however, their propagation below the estimated 16% maximum moisture content and 32°C. maximum temperature used in these tests is considered negligible. Such an estimation can be based on the works of Milner *et al.* (36), who recorded no decrease in total sugar content below about 17% moisture level in hard red spring wheat upon 20 days' respiratory trials at 30°C. temperature.

⁵Atmospheric fog has a droplet size distribution between 5 and 50 μ as measured by the cascade impactor (37,38).

granule surfaces created by size reduction procedures for explaining the substrate change. During storage, the droplets deposited onto the cleaved starch surface cause hydration, the degree of which is dependent upon the size of cleavage and the droplet. The temperature gradient or any other energy source which changes the entropy level during this time causes local retrogradation of the hydrated portion (surface and depth). A certain amount of crust is formed which renders a portion of starch insoluble (39) and thus unavailable as a substrate in the procedure of maltose determination (17,40).

The mode suggested for the explanation of decreasing maltose values with increasing exposure time is supported by the observation of Alsberg, that Congo red dissolved in anhydrous glycerol does not stain injured granules, nor does it stain whole granules; after standing some time, however, the glycerol takes up water from the atmosphere and the injured granules begin to swell (31).

If the moisture droplets are deposited on an area of particle contact points or within their close vicinity, the local hydration and dehydration welds and cements the particles together at their contact points, causing the increase of average particle size as determined by the permeability method (Fisher).

With the given explanation one would expect that the rate of maltose decrease is insignificant in the storage of flours of relatively low moisture level if the atmospheric moisture is excluded, and the rate is higher with larger cleaved starch granule content (see Tables II, III, and IV). The maltose values as obtained over the relatively large scope of changes support the explanation of Mangels (41), Dads-well and Wragge (42), Sandstedt *et al.* (32), and Andrews and Bailey (43) that the condition of substrate, or rather the starch resistance, is the predominantly responsible factor in determining the diastatic activity (maltose) of a flour.

Variation of pH, Fat, Fatty Acids, and Iodine Value in the Five Test Systems, and Its Analysis. The findings of previous workers concerning the increase of *fatty acid* and *hydrogen-ion concentration* with increasing storage time are confirmed by the data of this study including the lowering of pH with increased chlorine application (9,12) and the further pH decrease in subsequent storage (9). The data indicate that there are different amounts of fatty acid content in samples of equal age and of identical parentage. Generally the amount is dependent upon the specific surface (Tables II and III), exposure to different gases (Table V), exposure to different types and degrees of size reduction and/or agitation (Tables IV, VI, VII, and VIII), exposure to higher temperature level in rapid agitation (Tables VI,

VII, and VIII), and upon multiple contact exposure to metallic surfaces. *The gradient of the fatty acid content during storage* increases with higher moisture content as recorded previously in the literature (2,4,5,6,30) and can also be observed by comparing the corresponding data in Tables II and III; generally it increases with smaller particle size, or rather with higher specific surface (see Tables II and III); more specifically it follows the total starch granule surface in the flour samples (44). It is larger if the multiple impact is done in rapid air suspension as compared to that in a ball-mill (Table IV); it is considerably lower if the sample was handled previously with chlorine gas; it is lower if the sample was handled in rapidly agitated air suspension previous to the storage, and again higher if the rapid agitation in air suspension is done at a higher temperature level (see Tables VI, VII, and VIII). The amount of *petroleum ether-extractables* generally follows the protein level in the samples (28), but slightly decreases upon exposure to rapid agitation in air suspension and decreases more if the air is heated (see Tables VI, VII, and VIII), or if the flour is exposed to metal surfaces (see Table IX). Accepting that a lower *iodine* value of the petroleum ether extract measures the oxidative type of lipid decomposition *in situ* or rather the degree of oxidation, it appears that a relatively large amount of energy is required in terms of exposure and storage to oxidize the flour lipids *in situ*, except if chlorine or other reactive gases are used (12) or metals catalyze the decomposition. However, if the latter conditions are available, the lipids in a flour may become heavily oxidized, as the low iodine values in Tables VI, VII, VIII, and IX indicate.

Analysis. The discussed lipid data strongly suggest that the aging phenomena within flour samples are closely tied to the lipids and to their distribution or rather availability for exogenous energy sources in exposures. The aging process is extremely complex. The lipid loss in a rapidly agitated air suspension and increased loss in a rapidly agitated hot air suspension can be explained in that the flour particles, especially the starch granules (see Table VIII), are exposed to elastic deformation upon impacts in rapid succession, which promotes the disentanglement of large lipid molecules from their molecular bondage where they were naturally deposited in the ripening grain. While such disentanglement is proliferated mainly by overcoming the secondary valence forces (clathrate, Van der Waal, electrostatic), in the case of relatively large molecules primary bondages may well be involved. Volatile products of fat decomposition having low molecular weight such as small-chain fatty acids, ketones, aldehydes, and carbonyls may become air-borne, depending on their physical location (close regions

of the particle surface), on the frequency of impact, and on the amount of energy applied; thus the collected flour particles will yield less extractable lipids. Depending on the intensity and kind of exposures, more or less fatty acid may be lost and perhaps produced, explaining the different amounts reported in the five series.

The slightly decreasing tendency of petroleum ether-extractables with increasing storage time prolonged up to 22 years was observed also by other workers (6,45). However, the rather large loss of petroleum ether-extractables during prolonged storage attended by low iodine values (Tables VI, VII, and VIII) can be explained either by the extreme decomposition and evaporation of the nascent compounds (these samples had rancid odors) or by specific oxidative changes in fats during storage which render certain portions of lipids insoluble and unextractable by the solvent used.

Bungenberg de Jong *et al.*, reviewing the literature on the results of the exposure of flour-water mixtures to metallic surfaces such as can be observed in aluminum and copper bowls or bronze mixer (46) by "more oxidized" extensigraph character, list potential explanations of previous workers of the subject, all of which involve proteins and their oxidation systems (47). The milling experiment with steel and brass balls presented here suggests that the metallic exposure involves the catalyzation of lipid decomposition while it is still in molecular bondage of the flour particles, even in the absence of larger amounts of water such as in a dough mixer.

The catalytic decomposition of flour lipids *in situ* upon exposure to metallic surfaces may be considered identical to the rancidative decomposition of other fats such as lard upon the influence of metals as measured by Emery and Henley (48), except that the phenomenon is to be viewed in the light of the quasimolecular distribution of flour lipids among other flour constituents, as contrasted to the mass occurrence of fat molecules as in lard.

The apparent recovery of iodine values of the steel-ball-milled stock upon 20 weeks of storage is a surprise and may perhaps be explained in that, during storage at least, portions of the oxidized, decomposed lipids became air-borne and lost; thus the petroleum ether had access to the less decomposed portions of lipids which yielded extracts of higher iodine values. This perhaps could have been checked by acid hydrolysis data; however, the samples had been discarded and such data could not be made available.

The dependence of acidity on the moisture level and especially its time gradient during storage (1-7,49) and that of the iodine value of petroleum ether-extractables upon exposure to oxygen (5,10) or air

(see Tables VI, VII, and VIII) is shown also in the present tests. The mechanism of change may be explained in that at low moisture level the intermolecular passages for the access of oxygen to oxidizable portions of the complexes are not blocked, yielding low iodine values in the petroleum ether extracts. Through such open passages the products of the thermal lipid decomposition, among them short-chain fatty acids, are able to evaporate from the samples, yielding relatively lower fatty acid figures. However, at higher moisture level such passages may become blocked by water molecules, preventing the access of oxygen for the double bonds specifically located on the fat molecules, or rather in their complexes; in such case oxidation cannot occur, and the petroleum ether extract shows relatively higher iodine values. The lower iodine value of 43 measured by Halton and Fisher (10) at the high moisture level of 20% in a sample stored for 97 days at 60°C. under oxygen may be explained thus: that in an excess of water, hydrolytic scission of lipid and other molecules could occur at such high entropy level (60°C.) because of a rather large excitation of molecules, i.e., movement in relation to each other, permitting the oxygen to reach the specific location of double bonds in the molecular complex of the flour particles. At this position the oxygen can become part of the reaction called autoxidation, in which the double bond becomes excited upon capturing energy from outside sources such as are listed and used in the present tests. If the energy of the bonds reaches a critical level, the excess energy is dissipated by the electron breaking away from the rest of the molecule and taking a proton with it. In other words, the electron possesses enough energy to overcome the binding force between the carbon and hydrogen atoms, leaving the fatty acid molecule with a carbon atom containing an unpaired electron. Such a structure is called a free radical and has an extremely unstable chemical structure ready to further reactions (50).

The autolytic degradation of lipids (fats and lipoids) in flour is believed to be caused by enzymes contained by the flour itself (51-54). Such degradation assumes "contact" of the active chemical groups of the enzyme with the "available" portion of the substrate (35,55). The degradation of flour lipids *in situ*, as observed and indexed in the present studies, may be explained *with* or *without* the activity of lipolytic enzymes contained by the samples. The energy supplied by the temperature gradients in storage by the cyclic expansion and contraction of the stored matter and/or by the different exposures, as described previously, may be sufficient to move into close contact the chemically active portions of relatively large molecules, or it may also be sufficient to open clathrate bondages or to break other second-

ary and even primary bonds without the interference of an organic or metallic catalyst. Indeed, the former suggestion can be sustained only with the assumption that the "active spots" are naturally deposited or arranged relatively close to each other and that in the arrangement the presence of water plays a vital role.

In view of the suggested explanation, it is not unreasonable to speculate that the lipolytic activity of flour as measured by Sullivan and Howe (52) (0.5 g. flour, 10 cc. water, 2 cc. toluene, 37.0°C. temperature, 24 hr. incubation time, individually a long series of 1 g. fat substrate, and acidity level expressed in cc. of 0.1N sodium hydroxide, at different pH levels in the 7.6-5.2 range) was not caused only by the special action of lipases contained by the flour samples but rather by the specific exposure in the tests as described.

It is possible that the availability (access and blocking) of excess energy for the excitation of double bonds beyond the critical limit at which an electron can break away to produce free radicals and further decomposition of fats is governed primarily by the described procedure, by the specific porous properties of the individual flour samples, and by the specific properties of fat substrates used in the tests as compared to the innate lipolytic enzymes of the samples, which will have to be liberated from their bondage and transported to the vicinity of reactive loci of lipid molecules before they can reduce by virtue of their stereospecificity the energy required for a reaction to take place (50).

Former findings, that the lipid protein ratio is larger in air-classified low-protein fraction as compared to that in the high-protein fraction both of the same parental origin (12,56) and interpreted that the endosperm lipids as integral parts of a previously living organism are concentrated at certain biologically strategic locations of the endosperm (12,44,56) together with the involvement of lipids into flour aging phenomena as demonstrated by lipid indices in the present study, suggest that the endosperm lipids do not occur in the flour in the form of globules, i.e., in a conglomeration of many lipid molecules, as can be shown by microscopic techniques in aleurone (57). Rather, their distribution follows a quasimolecular pattern in which the lipid molecules are entangled in certain configuration with neighboring protein, carbohydrate, or other molecules, forming chemical complexes with them, as suggested also by Sullivan (54). The lipid molecules or a portion of them may be disentangled, dislodged from their clathrate or other bondage at different speed and at different degree dependent upon the condition of the flour and upon the dislodging method applied. They are subject to decomposition like any other

known fats or lipoids if they are liberated or partly liberated from their natural bondage and are thus rendered available for decomposition or any other change including oxidation.

Thus in the experiments of Smith and Andrews (15), flours stored at -20°F . did not take up more oxygen with longer storage time, as the flour was not exposed in any considerable degree to changes at this relatively low entropy level. However, if stored at room temperature conditions, i.e., at a higher entropy level and also at a larger degree of temperature alternation, the oxygen uptake in subsequent mixing increased with longer storage time to a maximum, as measured also by Cosgrove (16). In other words, at the higher room temperature level and at the alternating temperature (and atmospheric conditions), more lipid molecules became dislodged or partially dislodged with longer storage time, and made available for oxygen uptake in subsequent mixing; whereas at the -20°F . temperature level very little or no energy was available to dislodge or partially dislodge lipid molecules from their natural bondage which could take up the oxygen.

Halton and Fisher (10) were able to decompose lipids in flours; i.e., to oxidize the petroleum ether-extractables to lower iodine values of 56-76 by exposing them to oxygen in sealed containers at 60°C . in presence of solid caustic soda and 20% caustic soda solution (decreasing and increasing the moisture content of the samples respectively) and chloroform vapor for 39 days. They also found in a 128-day storage experiment that oxidation at 60°C . takes place most readily with flours of abnormally low and high moisture contents and the oxygen absorption is accompanied by an oxidation of flour fat (low iodine value). These observations led them to conclude that the flour fats are responsible for oxygen absorption in flours. However, if flours are exposed to oxygen in a cursory manner, such as in a tumbling box, for only a few minutes' duration (Table X), instead of the 128-day storage at 60°C ., in lack of sufficient energy to dislodge the lipids from

TABLE X
ANALYTICAL DATA OF A SOFT AND A HARD WHEAT FLOUR EXPOSED TO OXYGEN IN A
TUMBLING BOX AT ABOUT ATMOSPHERIC PRESSURE

FLOUR	OXYGEN FORCED INTO BOX	MOISTURE	MALTOSE	AWRC	FAT EXTRACTED BY PETROL ETHER	FATTY ACID (FAT BASIS)	IODINE VALUE OF PETROL ETHER EXTRACT
	cc.	%	mg./10 g.	%	%	%	
k) Soft wheat	0	9.8	119	..	0.68	32.0	107.5
	5,000	10.3	114	..	0.67	33.6	106.2
l) Hard wheat	0	12.0	163	63.9	0.80	13.8	112.1
	5,000	12.3	157	65.5	0.78	13.7	112.8

the molecular bondage practically no oxidation of the petroleum ether-extractables occurred.

Dörner (2) measured by extensigraph an "oxidative type" of changes in petroleum ether-extracted and stored flours, similar to changes recorded in unextracted flours. On the basis of such observations he concluded that the flour lipids have no "deciding" effect upon flour-aging phenomena. This conclusion may be interpreted in the context that the lipid decomposition, or rather their decomposition products, indicate a certain disintegration within the molecular structure of the proteinaceous matrix and/or starch granules of the flour particles as measured, indexed, and explained in the past and present papers, including some of his own data, and which have obvious rheological consequences as reported in his work.

The alkaline *water retention* capacity (21) is difficult, if at all possible, to interpret with the rather drastic interference into several flour components. In a vague manner it measures the hydration capacity of a sample in an excess of water at a chosen condition. Stated another way, it measures its capacity to withhold the adsorbed/absorbed water as against a standardized compressing force in the centrifuge. The relatively greatly increased withholding capacity during abusive storage of the ball-milled low-protein fraction (Table IV) can be explained in that the water absorbed in the soaking period by the starch granules and by the cleaved-crusted granules can be drained from their bondage only to a limited extent by the squeezing limited to a singular direction, or rather by elimination of a portion of intermolecular pores in the hydrated portions through the pressure in the centrifuge by the elastic contraction of the unhydrated starch granules or unhydrated portions of starch granules. It is also quite possible that the not-retrograded portion of starch granules, once hydrated, holds onto the water to a certain extent mainly by secondary valence forces.

The analysis of the data indicates that next to flour storage there are numerous different means⁶ that can bring changes in the entropy level of a flour and thus modify its properties. The relatively large gradient of the surface, maltose, lipid, and some hydration indices used in the present study strongly suggests that the rheological studies

⁶Uchino and Whistler were able to show that upon 3 days' exposure to light a larger amount of chlorine could be adsorbed/absorbed at 25°C. by commercial wheat starch extracted by 85% methanol for 2 days than by a similar sample in the dark (58). Whelan and Peat (59) distinguished between photo-oxidation of starch and allied carbohydrates as in the presence and absence of oxygen. They found that the degradation products in nitrogen did not contain the degradation products obtained in oxygen and offered a marked resistance to subsequent photo-oxidation. Emery and Henley recorded the decomposing potential of light upon fats and found that metals and light acted equivalently in the presence of oxygen to develop fat rancidity (48). Such observations suggest that the energy-causing changes in starch (and/or flour) samples during their history have many sources including radiational sources, and encourage food for speculation whether or not residual lipids are involved in the mechanism of changes recorded by Uchino and Whistler.

searching for fundamentals and for the effect of reducing and blocking agents should lean heavily upon the history of the samples; i.e., careful selection of the test flour, detailed description of its condition, and flour properties partially described and/or interpreted by the indices used in this study.

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