Effects of Moisture and Temperature on Microbiological and Sensory Properties of Wheat Flour and Corn Meal During Storage[1]

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ABSTRACT

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Wheat flour and corn meal were stored at moistures ranging from 11 to 18% and temperatures of 25 and 34°C for 18 months. Samples were assayed at 0, 0.5, 1.5, 3, 6, 9, 12, and 18 months. Microbial quality was monitored during storage by determining CO_2 in the headspace of each storage container and the total aerobic bacteria and mold counts. A sensory evaluation was made on each product. In general, bacteria did not grow under the relatively dry conditions used in this study, and their numbers diminished with time. However, mold counts increased in products stored at 15 and 18% moisture, reaching maximum numbers by three to six months, and then decreased during the remaining storage. *Aspergillus glaucus* and *A. candidus* were the predominant molds observed. Generally, increases in CO_2 in the headspace paralleled increases in mold counts and could be used to detect early microbial activity. High CO_2, high mold counts, and low flavor and odor scores were associated with storage of both products at 15–18% moisture and at 25 and 34°C. This study indicates that product quality cannot be maintained beyond six months at 15% moisture and 25°C or beyond 15 days at 18% and 25°C. Quality deteriorates more rapidly at 34°C than at 25°C at these moisture levels.

Meals and flours made from cereals ordinarily are not processed to greatly reduce their natural microflora. Consequently, these products are likely to contain molds, yeasts, and bacteria, which will grow if enough moisture is added. A little moistening will permit growth of molds only, whereas more moisture will allow yeasts and bacteria to grow (Frazier and Westhoff 1978).

Usually, the natural microflora of corn meal and wheat flour do not constitute a spoilage problem in themselves because moisture content is too low to support even the growth of molds. However, when these products are used in the formulation of certain foods, storage temperature and moisture may be critical factors affecting quality. For example, if the food industry should desire to market products containing mostly corn meal and/or wheat flour at moisture levels above the normal 11–12%, the storability of the products would probably be affected. Therefore, we undertook this study to define more precisely the moisture and temperature limitations for storing corn meal and wheat flour.

MATERIALS AND METHODS

Bleached all-purpose wheat flour and yellow corn meal were purchased from a local market. The moisture content of the flour and meal was adjusted to approximately 11, 13, 15, and 18%. After moisture equilibration, 250-g samples of material were placed in 16-oz bottles, which were tightly capped. The samples were stored at 25 and 34°C. At least eight bottles at each temperature-moisture combination were prepared to provide samples stored 0, 2, and 6 weeks and 3, 6, 9, 12, and 18 months. Moisture was determined by a vacuum oven method (AACC 1962). Carbon dioxide was determined by analyzing the headspace gas by gas chromatography as described by Ramstack et al (1979). Total molds and total and kinds of aerobic bacteria were determined according to procedures outlined by Bothast et al (1974).

Sensory evaluation was performed by a 12-member panel familiar with flavor characteristics of cereal products. The samples were presented to the panel as a cooked gruel. Unaged controls were also given to the panel at each testing session. Panelists were asked to rate the intensity of flavor and odor on a scale of 10 (bland) to 1 (very strong). Any samples having mold and yeast counts greater than 10^4/g or high CO_2 production were not tested, and as soon as a sample reached a flavor score of 5.5 or less, it was not tested at more severe storage conditions. For purposes of statistical analysis, the untested samples were given an arbitrary flavor score of 5. Flavor scores at the more extreme conditions were examined relative to other product measurements. All moisture levels were compared in the same test for each combination of time and temperature of storage.

Each product measurement was analyzed by analysis of variance. Variations attributable to main effects—storage time, moisture level, and temperature—and to their interactions were estimated and tested for significance. In addition, equations were fitted to the data, using time of storage and moisture as independent variables. Figures prepared from these equations were also used to examine effects, but are not shown.

RESULTS AND DISCUSSION

A summary of the various measurements appears in Table I. Means associated with levels of the three factors and the least significant differences at the 0.05 level are shown. Because no significant interactions were found except for CO_2, these means effectively indicate results. CO_2 showed a significant interaction between initial moisture and storage time. Because a wide range in values was observed, means were based on an analysis of logarithm of the percent of CO_2, and the antilogs of the means are shown in Table II. The least significant ratio at the 0.05 level is also shown for each product. Ratios of two means exceeding the least significant ratio are significant.

Moisture Effects

Variable moisture levels of the flour and meal were attained by adding the calculated amount of water needed to achieve the desired moisture content and, after a period of equilibration, determining the actual moisture. In the wheat flour samples, actual moisture content generally was higher than the calculated moisture at the 11% level, whereas at the 13, 15, and 18% levels, actual moisture was usually less (Table I). Similar results were noted with moisture contents of the corn meal.

Significant variation in CO_2 content, bacterial counts, mold counts, or odor and flavor scores at 25°C for both wheat flour and corn meal was associated with initial moisture level (Table I). At 11% moisture, a negligible increase in CO_2 at 18 months of storage was found (Table II). At 13%, corn meal showed an increase in CO_2, whereas wheat was relatively unaffected. At 15%, corn meal yielded higher CO_2 levels than those of wheat. At 18%, both products had increased CO_2 at two weeks.

The 15% moisture level appeared to be the breakpoint. Deterioration began at as early as nine months with wheat flour and six months with corn meal. CO_2 began increasing after 1.5

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TABLE I
Summary of Meal Characteristics of Products Stored Under Varying Conditions

	Corn Meal						Wheat Flour					
Conditions	Actual Moisture (%)	CO_2 (%)	Bacteria (log of TABC[a])	Mold (log of TMC[b])	Flavor Score	Odor Score	Actual Moisture (%)	CO_2 (%)	Bacteria (log of TABC[a])	Mold (log of TMC[b])	Flavor Score	Odor Score
Calculated moisture, %												
11	11.2[c]	0.03	2.31	2.39	7.5	7.4	11.3	0.03	2.51	2.27	7.5	7.6
13	12.9	0.17	2.34	2.27	7.2	7.3	12.2	0.05	2.45	2.16	7.4	7.4
15	14.5	1.77	2.23	3.17	5.9	6.0	14.1	0.58	2.57	2.24	6.0	6.1
18	16.8	3.48	2.83	4.65	5.4	5.4	16.7	2.03	2.79	3.93	5.3	5.4
LSD	0.3	···	0.52	0.83	0.4	0.2	0.3	···	0.45	0.70	0.5	0.4
Temperature, °C												
25	13.8[d]	0.28[e]	2.56	3.31	6.8	6.8	13.6	0.16[e]	2.75	2.84	6.8	6.8
34	13.9	0.60	2.30	2.92	6.2	6.2	13.6	0.25	2.41	2.46	6.3	6.4
LSD	0.2	···	0.37	0.58	0.3	0.1	0.2	···	0.32	0.49	0.3	0.3
Storage time,[f] months												
0	13.7[f]	0.02	3.36	3.00	8.1	7.5	13.5	0.02	3.72	2.62	7.6	7.6
0.5	13.5	0.15	2.70	2.29	6.9	7.1	13.3	0.11	3.20	2.24	7.2	7.3
1.5	13.8	0.29	2.51	3.72	6.6	6.7	13.9	0.06	2.56	3.46	6.8	6.9
3.0	13.7	0.71	2.06	3.69	6.6	6.6	13.9	0.32	2.34	3.61	6.6	6.6
6.0	13.7	0.82	2.08	3.50	6.0	6.0	13.5	0.35	2.38	2.40	6.5	6.5
9.0	14.2	0.96	2.38	2.80	6.1	6.2	13.3	0.34	1.75	2.63	6.3	6.3
12.0	14.2	0.74	2.27	2.48	6.0	6.0	13.7	0.80	2.08	2.32	5.9	6.0
18.0	13.9	1.98	2.08	2.45	5.8	5.9	13.3	0.59	2.60	1.89	5.7	5.9
LSD	0.5	···	0.74	1.17	0.6	0.3	0.4	···	0.64	0.98	0.6	0.6

[a] Total aerobic bacteria count per gram.
[b] Total mold count per gram.
[c] Each mean computed from 16 observations of temperature and storage time.
[d] Each mean computed from 32 observations of moisture and storage time.
[e] A significant temperature difference for corn meal but not for wheat flour.
[f] Each mean computed from eight observations of moisture and temperature.

TABLE II
Mean Percent of CO_2 Level in Headspace During Storage of Cereal Products at 25 and 34°C

	Moisture, %			
Storage Time	11	13	15	18
	Corn Meal			
0	0.02[b]	0.02	0.02	0.02
0.5	0.02	0.04	0.10	6.27
1.5	0.02	0.04	2.49	2.85
3	0.02	0.23	6.14	7.49
6	0.03	0.40	5.76	6.53
9	0.03	0.49	6.30	9.01
12	0.02	0.16	8.38	10.17
18	0.05	2.54	9.13	12.74
LSR[a] = 6.0				
	Wheat Flour			
0	0.02[b]	0.03	0.02	0.02
0.5	0.03	0.03	0.04	4.95
1.5	0.02	0.02	0.38	0.10
3	0.04	0.02	2.48	4.09
6	0.03	0.03	2.17	7.55
9	0.02	0.04	1.78	8.06
12	0.04	0.39	3.74	7.68
18	0.07	0.10	1.26	14.34
LSR[a] = 7.9				

[a] Least significant ratio at $P = 0.05$. Ratios of two means exceeding this value are significant.
[b] Values are the antilogs of logarithmic means.

months of storage in both samples. Both bacteria and molds decreased in the wheat flour, but counts in the corn meal increased somewhat during the middle of the storage period before finally decreasing. At 34°C, CO_2 showed considerable increases beginning at 1.5 months in both samples, and odor and flavor scores were unacceptable at three months for wheat flour and 1.5 months for the corn meal.

At 18% moisture, increase in CO_2 content in headspace gas was detected in both the wheat flour and corn meal samples and at both temperature levels within a two-week period. The increase continued with extended storage time. Mold counts increased in both the flour and meal at both temperatures, whereas bacterial counts increased only at 34°C. After two weeks, flavor and odor scores from wheat flour and corn meal at 25 and 34°C were all 5.0, indicating that deterioration had occurred early in the storage period.

Temperature Effects

The effects of storage temperature on wheat flour and corn meal were minimal (Table I). CO_2 production was higher at 34°C than at 25°C for corn meal, but temperature was not significant for wheat flour.

Temperature effects were most evident at the 15% moisture level. Sensory evaluations showed deterioration beginning after 1.5 months of storage at 34°C on both wheat flour and corn meal but after three months for samples stored at 25°C. Although major differences were not found in bacterial counts, minor differences were noted in mold populations in the samples. Mold counts were slightly lower in samples stored at 34°C.

At the highest moisture level used (18%), deterioration at 34°C was noted in samples of wheat flour and corn meal at as early as 15 days. Mold counts increased, and no sensory evaluations were conducted because of excessive molding. At 25°C, the 15-day sample of corn meal was tested but gave only marginal flavor and odor scores. The 15-day sample of wheat flour from the 25°C series gave a low flavor score and a medium odor score. Both wheat flour and corn meal stored at 25°C became visibly moldy after 1.5 months of storage and were not evaluated organoleptically.

Time Effects

In general, bacterial counts did not increase in wheat flour or corn meal under the relatively dry conditions used in this study, and their numbers diminished with increasing storage time (Table I). Mold counts increased slightly, reached maximum numbers by three to six months, and then decreased during the remaining storage. The CO_2 content of the headspace of storage containers increased during extended storage time for both wheat flour and corn meal stored at 15 and 18% moisture (Table II). This increase paralleled the increase in mold counts during early storage. With respect to flavor and odor scores, storage time did not appear to be a factor in low-temperature storage of either product at 11 and 13% moisture. However, at these moisture levels, storage time might be a factor at 34°C; decreases in sensory quality were noted at the longer storage periods. However, because of excessive CO_2, these products were not tested. The early appearance of molds on flour and meal samples stored at the two higher moisture levels precluded sensory evaluation of most of the samples, making time effects difficult to ascertain.

Predominant Microorganisms

The predominant molds identified in both the wheat flour and corn meal were *Aspergillus glaucus* and *Aspergillus candidus*. A few species of *Pencillium* and *Fusarium* were also observed. A very small number of *Actinomycetes* were detected in a few samples, but no particular pattern to their occurrence was found.

CONCLUSIONS

This study indicates that the quality of wheat flour or corn meal cannot be maintained beyond six months at 15% moisture and 25°C or beyond 15 days at 18% moisture and 25°C. Quality deteriorates more rapidly at 34°C than at 25°C at these moisture levels.

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Extraction of Unreduced Glutenin from Wheat Flour with Sodium Dodecyl Sulfate

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ABSTRACT

Glutenin from wheat flour was almost completely extracted with 0.5% sodium dodecyl sulfate (SDS), pH 7.0, without prior reduction of its disulfide bonds. Wheat flour was first extracted with 0.05*M* sodium phosphate, pH 7.0, containing 0.5% SDS at a 1:20 flour/solvent ratio. About 77% of the total flour nitrogen solubilized into the clear solution. The remaining proteins in the residue, mainly glutenin, were almost completely extracted by the second extraction with 0.5% SDS, pH 7.0, by stirring with a Waring Blendor for 2 min. No foam was produced by this procedure. This extraction procedure may be useful as a simple and rapid method for separation of glutenin.

Wheat glutenin is a mixture of high molecular weight proteins containing interpolypeptide disulfide bonds. Preparation of purified glutenin is difficult because of its high molecular weight and insolubility in buffers commonly used for protein separation. The complete solubilization of native glutenin into aqueous solution is one of the most important problems in the studies on the structure and characterization of glutenin. Glutenin has been obtained by precipitation with 70% ethanol from whole gluten (Jones et al 1959) and by extraction with various solvents such as acetic acid (Bietz et al 1975), AUC-solvent (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide, Orth and Bushuk 1973), acetic acid plus mercuric chloride (Mecham et al 1972), and sodium dodecyl sulfate (SDS) plus 2-mercaptoethanol (Danno et al 1976). Because of possible contamination during extraction and purification, data on the subunit compositions of the separated glutenin reported in the literature are conflicting. In this article, a new procedure using SDS as a solvent for the extraction of the glutenin from wheat flour without prior reduction of its disulfide bonds is described.

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MATERIALS AND METHODS

Materials

Wheat flour experimentally milled to a 60% extraction from Canada western wheat (No. 1) was used in this study. Flours from U.S. hard red winter and U.S. western white wheat were compared with the above flour. Wheat flour was defatted by being extracted several times with dry *n*-butanol and then air-dried. SDS (99% pure) was purchased from Nakarai Chemicals, Kyoto. All other reagents were either of guaranteed reagent grade or of the best grade available.

Extraction of Glutenin

Defatted wheat flour (100 g) was suspended in 2 L of 0.05*M* phosphate buffer (Na_2HPO_4-NaH_2PO_4), pH 7.0, containing 0.5% SDS. The suspension was gently stirred with a magnetic stirrer for 50 min at room temperature and was centrifuged at $18,000 \times g$ for 30 min at 15°C. A clear supernatant was obtained and was designated Fraction A. The residue was dispersed in 80 ml of 0.5% SDS, pH 7.0, by stirring at high speed for 2 min in a Waring Blendor. The slurry was diluted with 300 ml of 0.5% SDS, pH 7.0, and then gently stirred with a magnetic stirrer for 20 min and centrifuged at $22,000 \times g$ for 30 min. The clear supernatant obtained was designated Fraction B. No antifoaming agent was