Microflora of Barley Kernels[1]

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ABSTRACT

Follstad, M. N. (University of Minnesota, Minneapolis) and C. M. Christensen. Microflora of barley kernels. Appl. Microbiol. **10**:331–336. 1962.—Numbers and kinds of microflora were determined in 160 samples of barley grown in different regions of the United States; microflora were more abundant in the grains grown in the central states than in those grown in the western states. During steeping and germination in micromalting equipment, the number of colonies of filamentous fungi increased from two to five times, colonies of yeasts from five to ten times, and bacteria from 50 to more than 100 times the numbers present in the grain before malting. Kiln drying according to a commercial schedule reduced the number of all types of microflora below the number present before kilning, but all were present in larger numbers in the kilned malt than in the original grain. In barley stored at room temperature and at a moisture content of 15 to 18%, members of the *Aspergillus glaucus* group increased with increasing time and increasing moisture content, and germination percentage of the seeds decreased. Stored free of storage fungi at room temperature, barley with a moisture content just over 15% retained a high germination percentage for 5 months, but at a moisture content of 16% the germination decreased to zero.

The present report deals with numbers and kinds of microflora isolated from five varieties of barley grown on adjacent plots in three localities of Minnesota; from 76 samples of malting barley grown in 11 states of the United States in 1959 and 85 samples grown in 8 states in 1960; from stained and bright samples of barley after steeping, germinating, and kiln drying under conditions that approximate those used in commercial practice; and from samples of barley stored for 1.5 to 6 months at moisture contents that permitted invasion by storage fungi.

Materials and Methods

Moisture content. Moisture was determined by the two-stage, air-oven method specified by the American Association of Cereal Chemists (1957), and is expressed on a wet-weight basis.

Germination percentage. Two-hundred kernels were placed on moist paper towels, wrapped loosely in waxed paper, incubated at room temperature, and the number of germinated kernels counted after 3 days; any kernels with roots or acrospire were rated as germinated.

Numbers and kinds of fungi on and within the grain. Five grams of grain were comminuted in 500 ml of sterile 0.2% agar solution for 1.5 min in a Waring blendor, placed in 45 ml of the same suspension, shaken 100 times (further dilutions, if desired, were made in the same way), 1 ml of one or more of the final suspension was put in each of three petri dishes, agar cooled to 50 C was added, the dishes were swirled to distribute the material evenly, the agar was allowed to harden, and dishes were incubated in the laboratory at room temperature. Colonies were counted after 3 to 5 days by inverting the dishes on the stage of a stereoscopic microscope, with a magnification of 10 × and transmitted light, and were identified after they sporulated. Rose bengal agar and acid potato dextrose agar were used for yeasts and filamentous fungi, and tomato juice agar and potato dextrose agar for bacteria.

To determine the number of surface-disinfected kernels yielding microflora, kernels were shaken for 1 min in 1% sodium hypochlorite solution, rinsed in sterile water, and 50 to 100 kernels cultured on one or more of the media listed above.

Micromalting. Micromalting equipment was used, in which the temperature of steep water and of the germination chambers, as well as the cycling, were automatically controlled. Each sample consisted of 60 g of grain, which was steeped for 48 hr at 15 C with a water change every 12 hr; germination was for 72 hr at 16 C; the germinated grain was dried for 12 hr at 35 C, 12 hr at 45 C, 6 hr at 55 C, and 6 hr at 65 C.

Storage of samples at different moisture contents. Samples of 250 g each were stored in desiccators containing sodium chloride to maintain a relative humidity of 75%, ammonium sulfate to maintain a relative humidity of 80%, and potassium chloride to maintain a relative humidity of 85%, as described by Winston and Bates (1960).

Rootlet weight. This procedure was developed by Kotheimer and Christensen (1961) to measure germination vigor; 300 kernels were chosen at random from samples after steeping, germinating, and kiln drying, rubbed gently on a screen to remove and separate the rootlets, and the rootlets weighed.

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RESULTS

Microflora isolated from five varieties of barley. Certain varieties of barley are consistently less stained or weathered than others. One of several possibilities is that certain varieties might be inherently more resistant than others to invasion by the complex of microflora associated with staining and weathering, although no consistent difference in this was detected by Kotheimer and Christensen (1961). To test this, five varieties were chosen: Oderbrucker, because in the opinion of many experienced men it is less susceptible to staining than other malting varieties; Moore, because it was known or thought to be very susceptible to invasion by certain of the microflora; Liberty, because in moist harvest seasons it has developed a gray color that seems to be associated with high microfloral invasion; Kindred, because for many years it was the predominant variety of malting barley grown in Minnesota and North Dakota; and N.D. B-112, because it was a new variety.

The number and kinds of microflora isolated from these five varieties at three stages of maturity, all grown at St. Paul, Minn., during 1959 are summarized in Table 1. The dilution cultures of grain in dough stages yielded only a few colonies, although *Alternaria* grew from a majority of the surface-disinfected kernels. Mature grain of Moore and B-112 yielded about three to four times as many colonies of yeasts and bacteria as did Kindred, and also yielded yeasts and bacteria from about three to four times as many disinfected kernels as Kindred.

The numbers and kinds of microflora cultured from grain of these same five varieties grown at St. Paul, Waseca, and Crookston, Minn., are shown in Table 2. Again, dilution cultures of Moore and B-112 yielded from several to 15 times as many colonies of bacteria and yeasts as did Kindred, and bacteria and yeasts grew from more surface-disinfected kernels of Moore and B-112 than of Kindred.

The numbers and kinds of filamentous fungi cultured from any one variety were approximately the same as those cultured from any other variety. The weather during the 1959 growing season at all three locations where these test plants were grown was relatively dry from the time the grain headed until it matured, and was not favorable to heavy invasion of the kernels by filamentous fungi; a large percentage of the mature kernels yielded *Alternaria* and *Fusarium* after surface disinfection, but as judged by dilution cultures only small amounts of these fungi were present. The differences detected in number of bacteria and yeasts might be sufficient to warrant further investigation of difference among varieties in susceptibility of maturing kernels to invasion by microflora, especially in those years or those areas where the weather is more favorable to invasion than it was where these varieties were grown in 1959.

Numbers and kinds of microflora isolated from samples of

TABLE 1. *Number of colonies per gram, and per cent of surface-disinfected kernels yielding fungi, of five varieties of barley cultured at milk stage, dough stage, and maturity*

Variety	Colonies per gram (thousands)					Per cent of surface-disinfected kernels yielding				
	Alternaria	*Fusarium*	*Helminthosporium*	Yeasts	Bacteria	*Alternaria*	*Fusarium*	*Helminthosporium*	Yeasts	Bacteria
Milk stage										
Kindred	1	0	0	0	1	58	18	0	0	0
Liberty	0	0	0	12	1	84	16	0	0	0
Oderbrucker	2	0	0	1	4	86	10	0	0	0
Moore	0	0	0	4	1	64	16	0	0	0
B-112	3	0	0	8	2	86	14	0	0	0
Dough stage										
Kindred	1	1	0	0	0	80	14	0	0	0
Liberty	4	1	0	2	16	94	18	0	2	14
Oderbrucker	2	1	0	2	3	86	16	2	0	2
Moore	2	2	0	1	11	74	26	0	4	0
B-112	3	1	0	2	17	96	22	2	4	0
Mature										
Kindred	8	2	0	12	800	76	50	8	4	12
Liberty	12	1	1	22	1450	96	40	2	8	16
Oderbrucker	4	3	0	19	1950	86	48	2	8	28
Moore	16	6	1	32	3400	90	44	10	10	44
B-112	3	2	0	36	2600	86	40	4	12	46

TABLE 2. *Number of colonies per gram, and per cent of surface-disinfected kernels yielding fungi, of five varieties from St. Paul, Waseca, and Crookston, Minnesota, cultured at maturity*

Variety	Colonies per gram (thousands)					Per cent of surface disinfected kernels yielding				
	Alternaria	*Fusarium*	*Helminthosporium*	Yeasts	Bacteria	*Alternaria*	*Fusarium*	*Helminthosporium*	Yeasts	Bacteria
St. Paul										
Kindred	8	2	0	12	800	76	50	8	4	12
Liberty	12	1	1	22	1450	96	40	2	8	16
Moore	16	6	1	32	3400	90	44	10	10	44
Oderbrucker	4	3	0	19	1950	86	48	2	8	28
B-112	3	2	0	36	2600	86	40	4	12	46
Waseca										
Kindred	8	3	1	42	150	94	32	8	8	10
Liberty	3	2	0	24	350	94	28	8	6	10
Moore	7	2	1	59	2250	86	32	2	14	38
Oderbrucker	6	2	0	19	1650	94	26	12	4	24
Crookston										
Kindred	3	2	1	12	425	86	10	0	8	4
Liberty	5	2	1	27	425	88	26	6	10	8
Moore	7	3	0	42	3750	96	32	10	16	46
Oderbrucker	4	2	0	19	2100	94	34	0	12	32
B-112	4	1	0	53	2250	94	18	2	16	20

barley grown in different areas of the United States in 1959 and 1960. Seventy-six samples grown in experimental plots in different states in 1959 and 84 samples grown in 1960 were tested for numbers and kinds of microflora; the results are summarized in Tables 3 and 4. All of these samples were of known and named varieties, but in most cases different varieties were grown in the different states; the results are grouped according to the states in which the barley was grown.

In general, the samples from Michigan, Wisconsin, Minnesota, and North Dakota yielded *Alternaria*, *Fusarium*, yeasts, and bacteria from a larger number of surface-disinfected kernels, and in dilution cultures yielded a larger number of colonies of these organisms than did the samples grown in Montana, Idaho, Washington, Oregon, and California. This supports the data of Kotheimer and Christensen (1961). The conclusion, of course, is that microfloral invasion of barley kernels is mainly a function of weather during the time the kernels are growing and maturing. Germination of all of these samples exceeded 90%, and germination of most of them exceeded 95%, but the germination tests were made by incubating the samples on moist paper toweling at room temperature; in our experience this sometimes does not indicate accurately how the barley will perform when steeped and germinated under malthouse conditions.

Micromalting. DeClerck (1957) stated that seed-borne microflora may grow during steeping and germination of barley, and asphyxiate the barley embryos. Evidence from cooperative work between the Malt Research Institute (1955) and several malting and brewing laboratories indicates that weathered barley often produced malt or beer with abnormal or undesirable characteristics. Kotheimer and Christensen (1961) found a much larger number of microorganisms in stained barley than in bright barley, after steeping and germination under conditions that resembled those used in practice, and also found the germination percentage and rootlet weight of the bright barleys to be greater than those of stained barley. Thus, the evidence to date indicates that at least some of the complex of microflora present on and in barley kernels may increase greatly during steeping and germination, that they may affect the percentage, speed, and regularity of germination, and they may influence the quality of malt and of beer.

A number of micromalting tests were done, of which the results of only two will be given. A bright barley, var Betzes, grown in Idaho, and a stained barley, var. Liberty

TABLE 3. *Numbers and kinds of fungi, per cent of surface-disinfected kernels yielding fungi, and germination percentage of 76 samples from the M.B.I.A. for the 1959 crop*

Location	Number of samples	Per cent germination	Per cent of surface-disinfected kernels yielding					Number of colonies per gram (thousands)			
			Alternaria	*Fusarium*	*Helminthosporium*	*Cladosporium*	Yeasts and bacteria	*Alternaria*	*Fusarium*	*Cladosporium*	Yeasts
New York	2	96	12	29	0	0	34	—	—	1	4
Ohio	2	97	25	13	1	0	15	1	1	1	10
Indiana	1	96	28	22	4	0	10	—	—	1	1
Illinois	1	92	8	12	0	0	10	—	—	—	64
Wisconsin	6	91	76	58	2	0	16	11	10	2	4
Minnesota	12	96	65	22	13	0	10	37	33	8	35
North Dakota	19	97	36	26	11	0	9	16	1	1	48
Montana	6	99	2	3	0	0	1	—	—	—	3
Idaho	6	97	1	2	0	0	1	—	—	—	—
Washington	9	99	2	9	2	3	5	—	—	—	14
California	12	99	0	1	0	2	0	—	3	2	—

TABLE 4. *Numbers and kinds of fungi, per cent of surface-disinfected kernels yielding fungi, and germination percentage of 85 samples from the M.B.I.A. for the 1960 crop*

Location	Number of samples	Per cent germination	Per cent of surface-disinfected kernels yielding					Number of colonies per gram (thousands)			
			Alternaria	*Fusarium*	*Helminthosporium*	*Cladosporium*	Yeasts and bacteria	*Alternaria*	*Fusarium*	*Cladosporium*	Yeasts
Michigan	10	98	75	20	6	2	4	2	1	3	241
Wisconsin	9	97	70	16	8	4	3	9	6	9	141
Minnesota	6	98	84	12	1	3	29	17	8	3	239
North Dakota	31	97	58	11	6	5	16	4	1	2	106
Colorado	10	99	38	7	0	13	3	1	—	17	70
Idaho	8	99	8	2	0	17	3	1	—	1	11
California	8	98	22	4	0	19	4	—	1	4	40
Oregon	3	99	4	4	0	10	1	—	—	—	—

grown at St. Paul, were chosen for comparison. Two samples of each were steeped, germinated, and kiln dried in the micromalting equipment, and the number and kinds of microflora determined before steeping, after germination (rootlet weight determined then also), and after kilning. The results are given in Table 5.

After steeping, the stained barley yielded about five times as many colonies of bacteria, yeasts, and fungi as the bright; after germination, about twice as many colonies of bacteria, 14 times as many yeasts, and 6 times as many filamentous fungi, and after kilning about 5 times as many bacteria, 8 times as many yeasts, and 10 times as many filamentous fungi as the bright barley. Rootlet weight of the bright barley after germination was about three times as great as that of the stained barley.

In this test, two varieties of barley were compared, as well as bright vs. stained; it would be desirable to have bright and stained samples of each of several different varieties that had grown in adjacent plots. Up to now such samples have not been available.

Several sets of samples of the five varieties tested for comparative numbers of microflora, of which the results are given above, were also micromalted, and the number and kind of microflora determined at different stages of the process. The results of one such test are give in Table 6.

No obvious relationship is evident here between rootlet weight and microflora, although all of these samples had more microflora and lesser rootlet weight than the really bright Betzes in the previous test. The variety Oderbrucker produced about double the weight of rootlets of the other four varieties, and in dilution cultures yielded about half the number of yeast colonies of the other varieties in all the stages of the malting process. Obviously, much more work will be required to clarify the relationship between the microfloral load of barley, the increase of given species of bacteria, yeasts, and filamentous fungi during steeping, and germination, survival after kiln drying, and quality of malt.

Storage fungi. Tuite and Christensen (1955) stored barley at moisture contents of 13.7 to 16.6% for 7 months at room temperature, and found a close correlation between increase in storage fungi and decrease in germination percentage of the seed. They also stored barley, almost free of storage fungi and inoculated with various species of storage fungi, at different moisture contents for 15 to 30 days, and concluded that storage fungi were mainly responsible for decrease in germination percentage of the barley. There is now abundant evidence from work with wheat and corn that, at moisture contents normally encountered in storage, the storage fungi, and not processes inherent in the seeds themselves, are responsible for decrease in germination percentage and associated deterioration processes (Papavizas and Christensen, 1960; Qasem and Christensen, 1960). It was thought that different varieties of barley might be differentially susceptible to invasion by storage fungi when stored under conditions that permitted the storage fungi to invade them.

Samples of the five varieties of barley (Oderbrucker,

TABLE 5. *Number of bacteria, yeasts, and molds per gram of stained and bright barley during micromalting*

Variety	Bacteria (millions)	Yeasts (thousands)	Molds (thousands)	Rootlet weight (mg)
		Initial		
Betzes	—	—	—	
Liberty	18	5	8	
		After steeping		
Betzes	18	93	2	
Liberty	89	445	13	
		After germination		
Betzes	87	150	7	175
Liberty	202	2050	40	56
		After kilning		
Betzes	54	15	6	
Liberty	140	134	54	

TABLE 6. *Number of bacteria, yeasts, and molds (colonies per gram in thousands) before and after steeping, after germinating, and after kilning, from five varieties of barley, rootlet weight after germinating*

Variety	*Alternaria*	*Fusarium*	*Cladosporium*	Total molds	Bacteria	Yeasts	Rootlet weight
							mg
			Before steeping				
Kindred	10	10	32	52	6,000	84	
Liberty	3	5	47	55	36,000	105	
Moore	8	7	60	75	41,000	160	
Oderbrucker	5	3	14	22	20,000	68	
B-112	8	6	12	26	11,000	120	
			After steeping				
Kindred	10	22	5	27	37,000	1,240	
Liberty	—	10	3	13	49,000	1,910	
Moore	12	14	25	51	41,000	1,940	
Oderbrucker	2	18	—	20	26,000	590	
B-112	6	9	7	22	38,000	2,200	
			After germinating				
Kindred	38	31	20	89	1,010,000	2,300	47
Liberty	12	19	108	139	780,000	2,110	44
Moore	8	24	42	74	1,410,000	3,000	51
Oderbrucker	1	39	—	40	970,000	1,940	92
B-112	9	10	18	37	1,350,000	2,760	44
			After kilning				
Kindred	25	29	10	64	23,000	850	
Liberty	8	18	9	35	23,000	1,180	
Moore	14	9	24	47	26,000	1,150	
Oderbrucker	6	20	12	38	23,000	430	
B-112	10	14	16	40	26,000	1,550	

Kindred, Liberty, Moore, and B-112) whose microfloral load during development of the grain in the field and during malting and brewing had been determined, with results as given above, were chosen for the tests. Samples of 250 g each were stored at relative humidities of 75, 80, and 85%, at room temperature. At intervals of 1.5 months they were tested for germination and numbers and kinds of storage fungi. The results are given in Table 7. There were no large or consistent differences between varieties, and so each figure is an average of the five varieties. Included in the table are data from a separate study now in process with storage of different kinds of agricultural seeds free of storage fungi but at moisture contents in the range where storage fungi normally would be active. Barley stored free of storage fungi for 5 months at a moisture content of 15.1% and room temperature decreased only slightly in percentage and speed of germination, although at a moisture content of 16.2% the germination percentage was zero after 5 months.

The principal fungi that invaded these samples of barley stored at moisture contents of 14.6 to 17.2% were of the *Aspergillus glaucus* group. At moisture contents of 14.6 to 15.1%, only conidiophores were formed, mainly on or growing from the embryo; at moisture contents of 15.2 to 16%, both conidial heads and perithecia were present, although the perithecia were not numerous; at moisture contents of 16.2 to 17%, perithecia covered the embryo and a few conidiophores were present. That is, if barley is encountered, as it occasionally is several months after harvest, with perithecia of *A. glaucus* on the embryo surface, we can be fairly certain that it has been stored for some time with a moisture content of over 15% and probably over 16%.

Discussion

The most important factor influencing the invasion of developing barley kernels by microflora appears to be high relative humidity or rainfall after heading of the plants. In 1959 several heavy inoculations of plants in experimental plots, with individual and mixed species of microflora, did not result in any great increase in invasion or staining, probably because almost no rain fell after the plants were inoculated. Many or most of the microorganisms encountered on and in barley kernels probably are almost universally present in the air during the growing season.

No large and consistent difference could be found among five varieties of barley in susceptibility to invasion by the most common kinds of microflora isolated from stained and discolored kernels, although more yeasts and bacteria were found in varieties Moore and B-112 than in Kindred.

During steeping and germination of barley, using micromalting equipment designed to reproduce closely the conditions maintained in practice, the filamentous fungi increased from 2- to 5-fold, the yeasts from 5- to 10-fold, and the bacteria from 50- to 100-fold or more. The major increase in filamentous fungi was during germination of the steeped grain. Stained barley bears a much greater load of microflora than bright barley, and this difference may increase during steeping and germination. Kiln drying reduced the numbers of all of these groups of organisms, but the population of all three groups was several times greater in kilned malt than in the original grain.

In storage at moisture contents of 15 to 18% at room temperature, increase in *A. glaucus* (the only storage fungus encountered in any numbers) was proportional to

TABLE 7. *Germination percentage and fungus population of barley stored with natural fungus flora and free of storage fungi at different moisture contents at 20 to 23 C*

Months stored	Relative humidity	Moisture content	Germination	Surface-disinfected kernels yielding *Aspergillus*	*A. repens*	*A. amstelodami*	*A. ruber*	*A. restrictus*	*Penicillium*
	%	%	%	%					
1.5	75	—	91	18	72	3			15
	80	—	87	22	173	18			26
	85	—	77	31	472	34			5
3.0	75	14.7	85	40	404	31			9
	80	15.6	76	50	1,800	173	18		17
	85	16.1	68	69	2,486	120	34		4
4.5	75	14.6	68	61	500	220	20	260	80
	80	15.2	36	76	2,880	1,580	260	180	
	85	16.3	27	91	3,000	1,780	840		160
6.0	75	15.1	32	84	2,240	1,100	320	480	200
	80	16.4	1	100	76,666	33,600	3,400	320	200
	85	17.2	0	100	98,600	48,800	6,600		14,000
Free of storage fungi									
2.0	75	15.2	97						
	80	16.1	98						
5.0	75	15.1	93						
	80	16.2	0						

increasing moisture content of the grain and to increasing time of storage. As *A. glaucus* increased, germination percentage of the barley decreased. Barley stored free of storage fungi and at room temperature with a moisture content of just over 15% retained a high germination percentage after 5 months, but stored with a moisture content of just over 16% for 5 months the germination percentage decreased to zero.

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