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FLORA OF CORN MEAL

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INTRODUCTION

Corn meal as it comes from the mill carries the mycelia of certain fungi which infect unground grain. In addition, numerous species of molds and bacteria, present in spore form as contaminations upon the surfaces of sound kernels or as saprophytes in partially spoiled grains, are recoverable by routine cultural examination of the finished meal. Many experiments, extending over several years and including the work of various members of the Microbiological Laboratory, show that certain groups of organisms are practically always abundant in such cultures. Other species are usually present, but in smaller numbers, and many forms are obtained occasionally as accidental contaminations. In undertaking to study this complex flora, it may be possible to determine by routine culture the species represented and something of their relative abundance in the sample, but the list so obtained gives little information as to the relative importance of the individual species as causes of spoilage in the product.

The culture media commonly used in such routine examination of foodstuffs present conditions for the growth of microorganisms which differ greatly from those found in corn meal. The nutrients used in preparing such media are selected because they are readily assimilable to most organisms. These nutrients appear in solution or in jelly-like masses which contain high percentages of moisture. Corn meal, on the other hand, presents a range of composition, according to Winton and his associates (*8*),[1] approximately as follows: Moisture, 10 to 18 per cent, but under usual commercial practices ranging from 12 to 15 per cent; protein, 5 to 10 per cent; fat, 1 to 5 per cent, according to the method of milling; nitrogen-free extract, including starch and sugar, 68 to 78 per cent. Of the nitrogen-free extract, sugars constitute perhaps 3 per cent, and gums and dextrin, some of which are readily fermentable, perhaps an equal quantity. In dealing with this product as a substratum for organisms, the percentage of water found is an important limiting

[1] Reference is made by number (italic) to "Literature cited," p. 188.

factor. Obviously this product, even at its maximum moisture content, presents a marked contrast to laboratory media as usually prepared. Nevertheless, corn meal has been so often found an unstable product that it is commonly milled only for consumption within a few weeks or by methods intended to eliminate the most readily fermentable portions of the grain.

Under ordinary conditions of handling, spoilage in this product appears in one of the following forms: Souring, rancidity, mustiness, the formation of clumps or balls, extensive concretions which may involve the solidification of an entire bag, or the formation of a hard, cylindrical outer mass with the center loose and mealy. Heating occurs only in the wettest samples. Much corn meal, if held beyond a very short period, develops a musty, moldy, or sour odor and shows occasional balls or masses of meal held together by mold, which bring about losses in palatability and market quality in the product. Such changes as rancidity and the formation of extensive concretions into moldy masses are so obviously due to high moisture content and involve such losses that they have been almost eliminated from commercial practice. When losses occur the meal is found to carry more than a critical moisture percentage. This may be due either to milling corn which is insufficiently dried or to the storage of the meal under conditions which will maintain a moisture content above the danger point. For the samples used in all series reported here this figure was approximately 13 per cent (*2*).

CULTURAL EXAMINATION

In routine cultural examination reported here, plain agar was used for bacterial counts, wort agar for mold counts, and dextrose-litmus shake agar to determine acid, gas, and anærobic growth. The presence of particular organisms was determined by the use of special methods on special media. Experimentation covered a range wide enough to justify the restriction of routine cultures to the media already noted.

After comparative study of many series of cultures, Table I is introduced as giving a group of cultural results fairly typical for commercial meal in sound, merchantable condition. The nine samples reported were purchased in different retail stores of Washington, D. C., during October and November, 1920. Four of them were yellow and fairly coarsely ground. The white meals were softer or more finely ground. All were bolted. All showed by microscopic examination traces of both bran and germ, although these portions were scanty in certain samples. The history of the samples was not obtained.

These samples were sound in appearance and odor. There was no evidence of the multiplication of microorganisms. Among the bacterial colonies micrococci, members of the mesentericus and of the colon-aerogenes groups were characteristically present. Special tests in cab-

bage juice showed, in four of the nine samples, the presence of lactobacilli with the morphology and cultural characters of the organism of pickle and sauerkraut fermentation. No bacterial colonies were obtained in plain agar from two of the samples. A duplicate of sample 9 proved equally negative. Mold colonies were obtained in all samples. These represented in varying proportions *Aspergillus repens* De Bary, *A. niger* Van Tieghem, *A. flavus* Link, Fusarium, various mucors, and unidentified colonies.

TABLE I.—*Results of cultural examination of commercial corn meals*

Sample No.	Bacteria per gram on plain agar.	Molds per gram on wort agar.	Bacteria per gram on dextrose-litmus agar.	Acid colonies.
				Per cent.
1	10, 000	10, 000	16, 000	50
2	5, 000	1, 000	.....	.....
3	55, 000	12, 000	42, 000	60
4	60, 000	10, 000	13, 000	50
5	70, 000	400, 000	10, 000	50
6	5, 000	20, 000	8, 000	60
7	.....	3, 000	.....	.....
8	10, 000	11, 000	4, 000	100
9	.....	5, 000	3, 000	30

A more extensive series of studies was conducted in cooperation with the Plant Chemical Laboratory of this bureau. The general results of this experiment are described elsewhere (*2*). In brief, during the spring of 1920, a series of bags of meal were prepared for this storage experiment from corn bought by the mill in the regular course of business. This grain, while sold as No. 2, was obviously wet and barely passable as a fair product. Infected and even badly decomposed ears were not uncommon among the ears of corn received in bulk. Although the lots of meal included were milled at water contents varying from 12.7 to 16.18 per cent, the conditions of storage were such that no spoilage determinable by the senses took place. Cultures were made from the meal as freshly ground in April, then, beginning May 5, once each week until July. In all these cultures no evidence of multiplication of either mold or bacteria was found. It was, therefore, possible to follow the relative numbers of viable organisms in the various groups from the time of grinding through the four months of storage.

In the freshly milled samples the average count of colonies of bacteria upon plain agar was about 1,000,000 per gram of meal, with variations from 600,000 to 1,600,000. Upon wort agar the count of mold colonies averaged about 100,000 per gram of meal, with variations in different samples from 70,000 to 160,000. Of the bacterial colonies observed about 60 per cent were acid producers.

For comparison a special series of samples were prepared by adding 5 per cent of meal made from corn markedly rotted with Diplodia and Fusarium. In the freshly ground meal of this series the bacterial count upon plain agar was about 2,600,000. The count of mold colonies upon wort agar was about 110,000. About 70 per cent of the bacterial colonies were acid producers.

After storage for approximately one month (May 20 and 21) samples from a particular lot of five bags of the regular meal showed an average count of 108,000 bacterial colonies and 15,000 molds. Samples from the same bags on June 30 showed an average count of 12,600 bacterial colonies, and 7,600 mold colonies. Without placing emphasis upon exact figures, these cultural results are fairly typical of the mass of figures obtained from cultures made weekly from representative samples involving the whole series of 88 bags of meal. These figures are readily comparable with those obtained from commercial samples (Table I). Discrepancies which occur may perhaps be accounted for by the fact that samples 3, 4, and 5 were evidently the product of local mills, sold fairly quickly after milling, while samples 2, 7, and 9 were clearly the product of special processes and handled under conditions involving much slower distribution.

In this lot of meal, therefore, the conspicuous change due to storage was the drop in the number of viable organisms to about 1 per cent of the original number of bacteria and perhaps 10 per cent of the original number of molds. The larger part of this decrease occurred during the first six weeks, with a slow reduction throughout the succeeding periods.

In connection with the study of these figures, data obtained by Thom and Stiles (unpublished) in examining Winton's (*8*) samples[1] in 1914 were restudied and compared with the results here considered. Winton's corn meal varied in initial moisture content from 19.27 to 10.79 per cent. In those lots of meal (A, B, and C) carrying moisture markedly above 13 per cent, the evidence of multiplication of molds and bacteria was clearly discernible. Musty odors and balls of meal held together by mold were present in every sample. In cultures, the count of colonies of molds and bacteria reached 13 million in the wettest lot. Of these several million were *Aspergillus flavus*. The predominant organisms were molds rather than bacteria, but there was fairly clear evidence of some bacterial multiplication at the higher water percentages.

In the roller-ground samples of lots D, E, F, which did not spoil and whose water percentage was near to or less than 13, the total counts found by Stiles approached very nearly those already given in this paper. These examinations began too late in the storage period to show that part of the bacterial flora which dies off rapidly. The stored samples still showed some acid organisms, but micrococci and aerobic spore

[1] Samples of the meal studied were examined bacteriologically by G. W. Stiles, formerly of the Bureau of Chemistry, and for mold activity by Charles Thom, then in the Bureau of Animal Industry (*8*, *p.* 25).

formers of the mesentericus group formed the majority of the bacteria obtained.

In the lots with moisture content decreasing toward 13 per cent there was progressive reduction in the number of active species of molds. Extensive experimentation showed clearly that *Aspergillus repens* was the agent which formed the balls of meal loosely held together with mold hyphae, which characterized meal containing barely enough water to start spoilage. In another series of experiments *A. flavus* began to be active only in samples containing about 16 per cent of water. Yeasts, mucors, and Penicillia were reported by Stiles only in the sample carrying about 19 per cent of water.

During the examination of the preliminary samples in the 1920 experiment, an effort was made to identify the groups or actual species represented. As a matter of routine, inoculations were made from each flask prepared for diluting plates (consisting of 5 gm. of the meal to 45 cc. of sterile water) into the following media: Plain milk, gelatin, and litmus lactose broth. Smears were also made on Endo's agar in each instance. In every case there was prompt coagulation of the milk, with extrusion of whey, but no digestion of curd. Pink rings formed near the surface. Gelatin was liquefied in every instance, and acid and gas formed in all broth tubes. Growth in Endo's media indicated the presence of *Bacterium aerogenes* Escherich. Further cultural studies showed that *Bact. aerogenes* was the predominant bacterial species present in all these samples. This predominance was maintained throughout the series of examinations made. Microscopical examinations of smears made in each case, however, showed the presence of spore-bearing bacteria, especially the mesentericus group, and micrococci of various kinds. Dextrose agar tubes often contained colonies growing deep in the media, indicating the presence of anaerobic bacteria. Yeasts were found in all samples, their growth being largely of the mycoderma type. The plates showed many mold colonies. Various mucors, species of Fusarium, *Aspergillus flavus*, *A. niger*, and occasional green Penicillia were observed. The species of molds present on the plates varied from period to period and with the sample. Molds were always more numerous on plates made from meal to which Fusarium and Diplodia had been added, but growth on these plates did not show dominance of these particular forms.

Evidence of the effect of bolting upon the abundance of organisms was furnished in the 1920 experiment by the examination of samples of two series of five bags each, representing a single lot of meal, one-half of which was bolted and the other half unbolted. The bolting to which these samples were subjected removed a considerable part of the bran but little of the germ from the meal. After one month of storage, the bolted meal showed an average of 34,000 bacterial colonies and 20,000 mold colonies. The unbolted samples showed 108,000 bacterial colonies

and 15,000 molds. This observation was confirmed by a restudy of Stiles's unpublished examination of Winton's (*8*) samples. Of every lot of corn handled, part was ground in a stone mill without sifting or bolting and part was carefully "degerminated" and "roller" ground. In the bolting process all of the bran was taken out, and many of the samples consisted almost completely of horny endosperm. In that part of this series made up of meals in which no multiplication of microorganisms occurred, bolting consistently reduced the cultural count of microorganisms below that of the stone-ground meal. Frequently the number found in the bolted meal was less than one-tenth of that in the stone-ground meal.

By removing the bran, bolting takes away the largest area of contamination with saprophytic organisms. The tip of the kernel and the germinal area carry the majority of the infections found in corn. Study of many samples of corn over a period of years shows that invasion of the germinal area by molds is not uncommon in corn which has not been fully matured or has not been promptly and thoroughly dried. Samples have frequently shown the invasion of the germ in every kernel by *Aspergillus repens*. Recently samples representing a bulk shipment have shown nearly every grain to contain one or the other of two species of Penicillium. Meal therefore may be so milled and sifted or bolted as to remove the larger part of all contaminations, as well as those mold infections which do not involve general disintegration. The cleaning process before milling removes the grains thoroughly rotted by Fusarium and Diplodia. Corn has still been seen going into the rolls of a mill in which the low grade of the stock could not have been concealed if it had passed through a stone mill without being bolted. The product, however, was going into human food without showing tangible evidence of the low quality indicated by the unground grain. In other words, the fractional milling of low-grade grain makes possible such separation as turns the infected portions of the grain into oil stock or cattle feed and the solid or horny portions which are less obviously damaged into meal.

The literature of maize deterioration is reviewed by Alsberg and Black up to 1913 (*1*). The activity of Fusarium and Diplodia as causes of rotting in ear corn was discussed by Burrill and Barrett (*3*) and that of Diplodia alone by Heald, Wilcox, and Pool (*4*).

More recently McHargue (*6*) has studied the activities of certain fungi and their relation to commercial conditions in the handling of the product. Excessive moisture in the grain is regarded as the limiting factor in most cases of such spoilage. The factor of temperature must not be overlooked. The moisture content limit may be materially increased during the winter without evidence of the activity of microorganisms. The agents of spoilage in all the cases under review were primarily molds. The results already given in this paper harmonize in general with those

of McHargue. It has been possible, however, to go farther and indicate more clearly the groups of organisms regularly present and to record the conditions under which certain of them become active factors in spoilage.

Routine mass or dilution cultures show that certain molds are recoverable from practically all samples of meal. Among these are *Rhizopus nigricans* Ehrenberg and some of the mucors which frequently overgrow plate cultures within two days of incubation, although they probably are present only in spore form in the meal. Syncephalastrum, belonging to the same group, is not uncommon. *Aspergillus flavus* and *A. niger* are only occasionally visible factors in the infection of the unground grains, but they always appear as rapidly growing colonies in the mass or dilution cultures made. The brown masses of *A. tamari* Kita are commonly found with *A. flavus*. *A. fumigatus* Fres. and *A. terreus* Thom are frequently present but are quickly overgrown by the more active species already mentioned. *A. repens*, though practically always present, can be found only by careful search in the presence of these rapidly growing forms.

Several strains of Penicillium are found in meal cultures. Penicillia of the group with submerged orange mycelia and of the Citromyces group are probably most common. *Penicillium expansum* Link is reported by McHargue. *P. oxalicum* Thom and Currie is found in many samples of meal, but rarely in miscellaneous cultural work. Strains related to *P. luteum* Zukal and *P. purpurogenum* O. Stoll are frequently present but usually indicate soil contamination rather than active growth in the corn or meal. One sample of corn rotted by a member of this series has been examined, but the conditions shown clearly indicated that the product had contained high percentages of moisture at the time the rotting occurred.

Colonies of Fusarium develop from almost every sample of meal. Infections of this group are so abundant that conidia or grains of meal containing living hyphae are rarely absent. Cladosporium and Alternaria are frequently found but represent spore contamination rather than infection. The other organisms observed in culture from time to time appear to represent excessive contaminations with spores due to unfavorable conditions in the handling of the product, or, in certain species, to actual infection of the grain locally by the mold.

The bacteria found in the fresh samples here considered were predominantly *Bacterium aerogenes*. Certain other organisms have been regularly obtained in culture. When the necessary moisture is present, souring is so characteristic of the product that Round and Gore (7) found the addition of 3 per cent of fresh meal an adequate starter to insure the dominance of lactic acid fermentation in potato silage. Lacto-bacilli were present in four of the nine lots reported in Table I. According to unpublished records in the Microbiological Laboratory, Round found organisms of this group abundant also in fresh meal, but occasionally

absent in old meal or meal made from old and thoroughly dried corn. Micrococci are constantly encountered in culture but have not been typed. Aerobic spore formers of the mesentericus group are always present, and in spore form they constitute the larger part of the living bacteria in some meals after long storage.

This was clearly demonstrated by a series of experiments upon the possibility of producing a sterile meal with steam, dry heat, or both (unpublished cultural results of Ruth B. Edmondson). The spores of this group survived more heating than could be applied under practical working conditions to the product. Aside, however, from meal so wet as to be unmarketable, these experiments show no evidence of bacterial activity. One sample of apparently sound yellow meal showed the presence of *Bacillus niger* Migula in such extensive numbers that masses of meal placed upon culture media were promptly overgrown and with the agar turned bluish black with this species. The meal was contributed by Dr. S. S. Adams, of Washington, D. C., who reported the feces of a child apparently well to have been blue when fed this meal.

When, however, corn or meal is bottled and incubated at laboratory temperature (20° to 30° C.), those species capable of developing under the conditions presented show active growth. In the authors' series such growth was not detected by physical appearance in products carrying less than 13 per cent of moisture. Certain stone-ground samples of Winton's series (*8*) showed some evidence of mold activity below that figure. Measurable changes in quality certainly occur in such meals during storage. Some experimental results have suggested the possibility that these changes in such meal are due to the distribution of infected material throughout the mass by the grinding of infected corn. This conflicts with the current trade belief that the natural enzyms of the germinal area are the chief causes of such deterioration, but reflects the findings of Hoffer (*5*) and his coworkers that even selected seed corn may be extensively infected. Examinations of commercial samples in the Microbiological Laboratory have shown extensive development of molds within the grain itself in corn of other than the higher grades.

In samples carrying 14 to 15 per cent of water the formation of balls and concretions in the meal begins to be evident. The principal agent in their formation appears to be *Aspergillus repens*, although many difficulties are encountered in fixing a minimum moisture percentage for the activity of this species. Changes involving the development of mold mycelium in the meal begin within the limit of 13 to 15 per cent of moisture. Incubation at 20° to 30° C. merely accelerates changes which would progress more slowly in colder places. Moist chamber experiments with meal inside this range of water content show the presence of active mycelia of more than a single species, but principally *Aspergillus repens*. When the percentage of moisture reaches 16, several species are clearly able to grow. Special studies with *Aspergillus*

flavus show that very little development of this species occurs below 16 per cent, but that from 16 per cent upward development of this species rapidly increases and the number of forms capable of growing rapidly rises. Among the characteristic saprophytic molds observed under these conditions, in about the order of their abundance under the conditions, are *Aspergillus repens*, *Aspergillus flavus*, *Actinomyces* sp., *Penicillium* sp. and *Citromyces* sp., *Fusarium* sp., *Aspergillus candidus*, *Aspergillus ochraceous* Wilhelm, *Aspergillus tamari*, and *Aspergillus niger*.

Bacterial activity appears to be a concomitant of the disintegration due to mold action in such rotting processes as this. As indicated by Bailey and Thom (*2*, *Table I*), active disintegration by molds is accompanied by an increase in the water percentage of the sample. Bacteria follow rather than initiate the process in the samples studied, thus becoming a small factor in the merchantable product.

Throughout this investigation a close correspondence has been observed between the flora of deterioration in unground corn and the flora of the milled product.

SUMMARY

In seeking possible causes for the well-recognized instability of corn meal, cultures show considerable numbers of molds and bacteria to be generally present. Among these the following species of molds were characteristic of many series of cultures: *Fusarium* sp., *Aspergillus repens*, *A. flavus*, *A. tamari*, *A. niger*, *Citromyces* (or *Penicillium* section *Citromyces*) sp., *Penicillium oxalicum*, *P. luteum* varieties, *Mucor* sp., *Rhizopus nigricans*, and *Syncephalastrum* sp., together with various yeasts and yeast-like fungi. Among bacterial groups, the colon-aerogenes group and lacto-bacilli were most abundant in fresh meal. Aerobic spore formers and micrococci were always present and persisted in the stored product.

Within the range of composition found in merchantable meals, no bacterial activity was detected. Only one grade of unbolted meal showed signs of mold development below 13 per cent of moisture. Above 13 per cent moisture, *Aspergillus repens* begins to be an active agent of spoilage somewhere between 13 and 15 per cent of moisture, varying with the form of milling practiced. Several other species of molds are active in meal containing 16 per cent moisture; and numerous forms, including some bacteria, develop when 18 to 20 per cent of moisture is found.

Many samples of corn are found to carry extensive infections with Fusarium, Diplodia, *Aspergillus repens*, or Penicillium, especially in the germinal area and in the tip of the kernel. These sections of the kernel are removed in varying degrees by different milling systems. The bolted meals examined show a corresponding reduction in count of viable organisms as shown by culture.

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