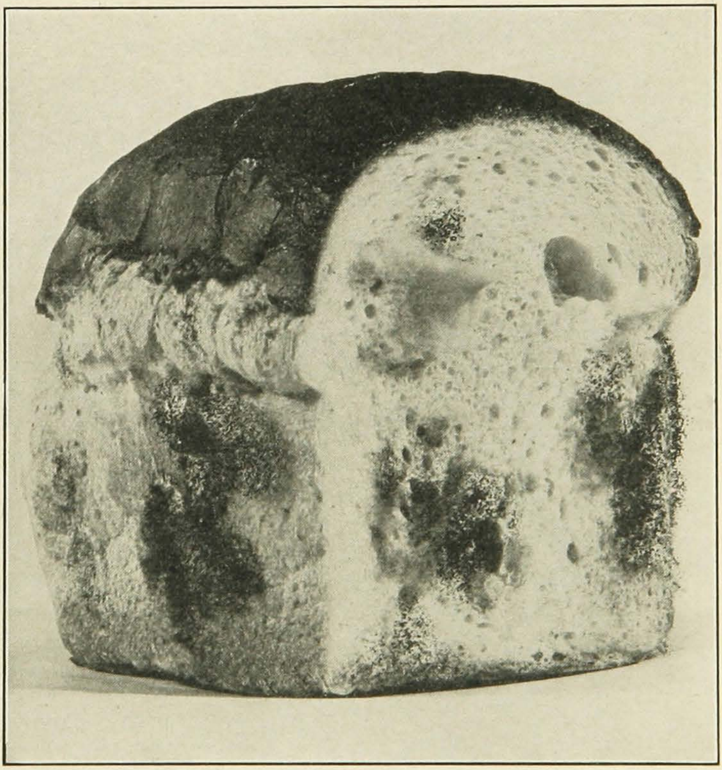
Bulletin 296 March 1933

UNIVERSITY OF MINNESOTA
AGRICULTURAL EXPERIMENT STATION

SOME NEW FACTS ABOUT MOLDS AND BREAD

OSCAR SKOVHOLT AND C. H. BAILEY
DIVISION OF AGRICULTURAL BIOCHEMISTRY



UNIVERSITY FARM, ST, PAUL

SOME NEW FACTS ABOUT MOLDS AND BREAD

OSCAR SKOVHOLT[1] and C. H. BAILEY

This is not just another discussion of the mold problem. If it were, its publication would probably not be justified, since much excellent general information is available on this subject. Characteristics of this fungus growth and methods for avoiding its development on bread have been the subject of many well written articles. Tho some of them have been presented in connection with some sales appeal this has not detracted from the value of the facts they have conveyed.

One might assume that a subject which has been so extensively discussed, would have been thoroly investigated. A review of available facts reveals that much is still unknown about the many factors which influence the rate and extent of mold growth on bread. Since the problem is of considerable economic importance to baker and consumer, additional time and effort have been devoted to an attempt to learn more about these factors. This treatise is not a repetition of general considerations, but is an attempt to review some of these recently discovered facts.

HISTORY

The bread mold problem was increased with the introduction of bread wrappers. Previous to that time, the protection afforded by low moisture content had usually been sufficient. Only an occasional instance of mold development was encountered, as when a housewife kept a loaf for a long period in a damp mold-infested bread box. Since neither baker nor consumer was willing to give up the sanitary protection that bread wrappers afforded, considerable attention was devoted to methods for the reduction of mold growth. Following this period there was a lag in investigational work tho frequent general reviews appeared in print.

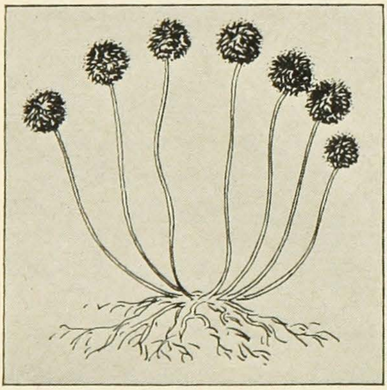
The last few years has seen a revived interest in the problem. This may have been largely caused by the general introduction of the practice of slicing bread. Again, we have a forward step from a convenience and merchandising standpoint that has undoubtedly been a factor in increasing losses due to mold.

[1] Research fellow of the American Dry Milk Institute.

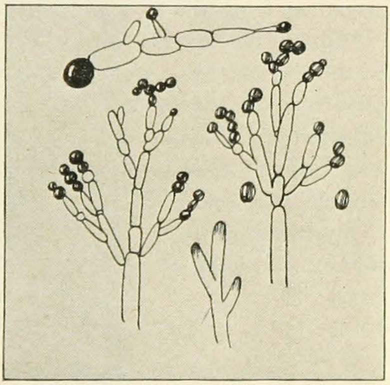
The authors desire to express their appreciation for the financial support of the American Dry Milk Institute in prosecuting researches in this field.

SOURCES OF MOLD SPORES

Bread that has been thoroly cooled and carefully wrapped under sanitary conditions is often found to develop mold within a few days. It may have been so handled that there were only two possibilities for contamination of the loaf. Spores may have been present in the ingredients and survived to infect the loaf, or may have been carried to the loaf through the air previous to its inclosure in a wrapper. Since mold spores as present in the air are invisible, many of them being as small as 1/10,000 of an inch in diameter, the possibility of contamination from this source is often under-estimated. This serves to direct suspicion to the dough ingredients as possible contributors of the undesirable organisms.



One Common Type of Black Mold Somewhat Enlarged
Reproduced by permission of The Mathieson Alkali Works.



Spores Borne on Fruiting Bodies of a Typical Blue Mold, Greatly Enlarged
Reproduced by permission of The Mathieson Alkali Works.

MOLD SPORES ARE KILLED IN BAKING

Many of the dough ingredients are known to be satisfactory media for mold growth and to contain spores which are only waiting for suitable conditions to bring forth innumerable mold plants. The question arises as to whether or not these spores are able to survive the fermentation and baking processes to reproduce their kind in the finished loaf. Some evidence has been accumulated on this point but, unfortunately, it does not lead to a verdict that is quite unanimous. One of the most careful attempts[2] at such a determination employed five methods of checking loaf sterility and these investigators concluded that a loaf of bread is sterile as it comes from the oven. Others have reached the same conclusion, but individual tests have not always been in agreement. Such disagreements have probably often been due to the difficulty of

[2] Prescott, S., Strider, J. W., and McClellan, R. N. Bak. Tech. 1:230-235. 1922.

insuring that contamination is not allowed after removal from the oven. A bit of additional evidence on the possibility of mold survival has recently been presented by the authors.[3]

In these tests active mold spores were introduced into doughs that were subjected to varying baking periods. The loaves were then cooled, hand-wrapped, and stored in a cabinet under room conditions. No attempt was made to attain sterile cooling conditions. The effect of oven heat on the contained mold spores was determined by comparing the resulting rate of mold growth with that on loaves which had been similarly tested, but without an addition of mold spores in the dough. The loaves that contained the previously added spores gave no evidence of increased mold growth over that shown by the uninoculated bread. In no case was there any evidence of growth in the interior of the loaf. The results seemed to justify the conclusion that bread which has received a thoro enough baking to be saleable will contain none of the spores of common bread mold having the ability to grow and reproduce.

INGREDIENTS AS BREEDING PLACES FOR MOLD SPORES

Evidence seems to assure that dough ingredients cannot directly contribute living mold spores to the finished loaf. How, then, can the dough ingredients selected for a particular bread formula be a factor in the mold problem?

First of all, let us consider a way in which they might have an appreciable effect. If reasonable precautions are taken in the matter of cleanliness of employees and apparatus, air must be the chief conveyor of mold contamination. Favorable breeding places for mold in and around the bread plant will greatly increase the number of spores in the air. Thus any ingredient that serves this purpose may have an effect on the extent of contamination. It is not likely that the presence and use of ingredients that are such contributors of mold spores will be permitted in a well-kept shop. But traces of such ingredients in inaccessible places, such as flour, dough, or bread crumbs spilled in cracks and corners, may readily have a tremendous effect in this way, even tho removed by quite a distance from the cooling bread. Stale bread, in the bakery or rooms of buildings in connection with the bakery, makes possible a live source of mold contamination.

BREAD AS A DESIRABLE MEDIUM FOR MOLD GROWTH

A second possible effect of dough ingredients would be their contribution to the nutrition and rapid development of molds growing on the resulting bread. No experiments appear to have been conducted that have aimed at the determination of the significance of ingredients in this

[3] Skovholt, Oscar, and Bailey, C. H. New South Baker. Oct. 1932.

connection. But molds are quite susceptible to changes in hydrogen-ion concentration. Within the limits used in commercial bread, increasing active acidity will give more favorable conditions for mold development.

Milk is the principal common dough ingredient to influence decidedly the hydrogen-ion concentration of bread. The low normal hydrogen-ion concentration and high buffering capacity of milk solids give to the finished loaf a reduced active acidity. This characteristic should tend to reduce the rate of mold development when milk solids are used, since molds flourish best in a slightly acid medium.

MOISTURE AND HUMIDITY CONSIDERATIONS

One of the essentials in mold growth is an ample supply of moisture. On the other hand, a baker cannot afford to let his bread dry out just because of that, since the average consumer prefers a fairly moist product. Bread crust is quite immune to mold attack if dry. This may be partially due to the physical difficulty molds have in penetrating this outer layer unless softened by moisture in the enveloping air. It has been found that the humidity surrounding the loaf has a more definite effect upon the rate of mold growth than the moisture content of the medium itself. Afer a period of time, this air humidity and moisture content of the outer crust tend to reach a definite equilibrium if the bread is in a confined space. But this equilibrium is not the same for all materials. This is just another way of saying that substances differ in hygroscopicity.

Tho a high humidity is known to favor mold growth, the humidity level necessary to maintain mold growth on bread was not known. Then, too, only limited studies had been made of the balance existing between the moisture content of bread and the humidity surrounding it. This balance might easily be shifted by various dough ingredients. Such a shifting might be more decidedly caused by milk solids than by any other common ingredient. These problems appeared important enough to merit some attention. A series of investigations, accordingly, was made to throw some light on the questions of the effect of various humidity levels on mold growth and the effect of skimmilk solids on bread hygroscopicity.

DETERMINING HUMIDITY REQUIREMENTS

Sections of bread crust were placed in four containers that were maintained, respectively, at four definite humidity levels. A period of 24 hours was allowed to reach an approximate equilibrium between the moisture content of the crust and the humidity of the air. One section of crust from each container was then taken for a moisture test and the others were inoculated with active mold spores. Species of the three molds, which have been reported as being the most common in growth on bread, were used. These organisms were *Penicillium expansum*, *Aspergillus niger*, and *Rhizopus nigricans*.

Preliminary tests indicated that a fairly high relative humidity was essential for mold growth on bread crust. Two experiments were then conducted in which inoculated bread crusts were stored for seven days in air with relative humidities averaging about 85, 88, 91, and 94 per cent. The average results giving extent of mold development during this period and moisture content of crust in equilibrium with these humidities, are given in Table 1.

Table 1

Humidity and Moisture Essential for Mold Growth on Bread Crust

Relative humidity of container	Crust moisture	Growth (7 days) *Aspergillus niger*	Growth (7 days) *Penicilium expansium*	Growth (7 days) *Rhizopus nigricans*
per cent	per cent			
85.2	19.8	Trace	None	None
88.2	22.8	Light	Trace	Trace
91.2	25.7	Heavy	Light	Heavy
93.9	28.4	Very heavy	Heavy	Very heavy

These tests emphasized the sensitivity of mold to moisture and humidity. The extent of growth designated as "trace" could only be detected with a magnifying lens after the seven-day period. Thus it is seen that no growth of commercial importance develops on bread crust if maintained in a relative humidity below about 90 per cent.

EFFECT OF MILK SOLIDS ON BREAD HYGROSCOPICITY

The humidity of the air between a loaf of bread and a wrapper impervious to moisture is dependent upon the moisture content of the bread and its hygroscopicity. It was possible to determine the effect of skimmilk solids upon the hygroscopicity of bread crust and crumb by determining their moisture content after a storage period in containers maintained at definite humidities. Loaves of bread were baked without milk and with 6 per cent dry skimmilk. Sections of crust and crumb from each loaf type were placed in each of four containers maintained at as many levels of humidity. Four days of storage were allowed to insure an equilibrium before moisture tests were made.

Bread crumb appeared to be somewhat more hygroscopic than bread crust. The comparisons of crust and crumb of milk-free bread with corresponding sections from bread containing milk solids, revealed small differences at all humidity levels. The differences ranged from 0.1 to 2.2 per cent moisture and all were in agreement that each comparison showed the higher moisture content in the bread containing skimmilk solids. Averages gave a 1.3 per cent and 0.6 per cent higher moisture content for crumb and crust, respectively, in the instance of milk bread.

This means that if both types of bread were wrapped at the same moisture content, the humidity would be somewhat lower in the air surrounding the milk loaf than in that between the milk-free bread and its wrapper. It also follows that milk bread could contain a higher moisture percentage than the milk-free bread without causing a higher air humidity in its surroundings. This is fortunate, since milk bread is commonly higher in moisture content than bread without milk owing to the increased water absorption and retention effected by the use of milk solids.

It has been quite definitely established that common mold spores do not survive the baking treatment. Thus the ingredients used can not directly contribute living spores to the finished loaf. The effect of dough ingredients on the specific nutritive properties of bread as a supporter of mold growth are quite indefinite. However, it is known that ingredients such as milk solids tend to make the bread a less favorable medium for mold growth from an acidity standpoint. It has also been shown that milk bread may carry a somewhat higher moisture content than milk-free bread and still maintain the same relative humidity in the surrounding air. These facts may be individually interesting but only become of definite value when the sum of all of them is obtained. What is the net effect of skimmilk solids upon mold growth on bread?

COMPARISON OF MOLD GROWTH ON MILK BREAD AND MILK-FREE BREAD

Procedure.—The most definite evidence on this point can be obtained by observations of rate of mold growth on milk-free and milk bread. Such evidence has been accumulated. The development of methods was largely accomplished in the laboratory. Artificial inoculation of bread with mold spores, kept in dried dextrin, was tried with considerable success as a supplementary procedure. Its advantage is in insuring the presence of a large number of mold spores, which also shortens the necessary observation periods.

Laboratory comparisons.—A few comparisons of rate of mold development were also made in the laboratory using several lots of milk-free and milk bread, including both laboratory loaves and fresh commercial loaves. The loaves were cooled, hand-wrapped, and stored under similar conditions. Artificial inoculation was used in a few cases. Sections of inoculated crust and crumb of the two types of loaf were also stored in containers under constant humidity conditions, as previously described. Results of these laboratory observations showed no measurable difference in the rate of mold development on bread with or without skimmilk solids.

Planning practical commercial comparisons.—These tests were only preliminary and served chiefly as an aid in developing procedure. Bakers would also be interested in results that are obtained right in the shop under practical working conditions. A survey revealed that the southern Mississippi Valley probably provides as favorable conditions for mold growth as any area in the United States. It also revealed that July and August are the months of greatest grief due to these organisms.

Plans were laid, therefore, for a thoro investigation during midsummer of 1932 and in the areas indicated. This investigation was aimed primarily at the accumulation of sufficient evidence to support a definite conclusion with regard to the effect of skimmilk solids in bread upon the rate of mold growth. It was also planned to reveal the influence of certain different bakery conditions and practices upon mold troubles.

Bakery service men of the American Dry Milk Institute conducted these plant tests. H. G. Brouillet devoted the most time to the project with C. A. McDuffee taking charge in one of the plants. Planning of the project, general supervision, and summarizing of results were under the direction of the authors. Excellent co-operation was extended by the management of five bakeries in the states of Mississippi, Tennessee, Arkansas, and Missouri who volunteered their plants for this investigation.

Storage places.—In plant No. 1, only a brief study was made and comparisons were limited to observations of rate of mold growth on bread, with and without milk solids, which had been handled in a normal way. In this case the loaves were stored in a room adjoining the bake shop. More extensive investigations were made in the other four bakeries. Because of the express desire to make these tests as practical as possible, vacant store buildings were secured for storage of the bread during the period of these investigations. Thus it is believed that the comparisons were conducted under conditions like those encountered in the usual retailing of bread in this area.

Variables included.—Specific information was secured on the effects on the rate of mold growth of (a) plant sanitation, (b) length of baking period, (c) methods and duration of cooling and (d) storage methods and conditions. In studying all these variables, breads with and without skimmilk solids were both included and similarly handled. Thus evidence of the effect of dry skimmilk upon the rate of mold growth was secured while determining the effects of other factors. Artificial inoculation with mold spores was resorted to as a supplementary procedure in some cases, but the greater part of the data were obtained by observing bread which developed mold growth after being infected by natural processes.

Scoring method and procedure.—Twelve wrapped loaves of bread were usually taken to represent each batch or variation in method of treatment. These loaves were divided into three or four groups and one group was examined each day, usually beginning on the second day of the storage period. Hand lenses were available for the detection of minute traces of mold development. A scoring method was adopted to indicate extent of mold growth. Ratings of 0, 2, 4, 6, 8, and 10 were used to represent, in that order, increasing density and extent of mold development. Thus the highest total score in a comparison means greatest growth and departs from the common practice of indicating the best rating for the bread involved.

A detailed discussion of all of the numerous data would be lengthy and probably tedious. We will limit ourselves to a summary of these findings.

Effect of protected cooling.—One of the most conclusive results was obtained in studying the effect of partial protection of the loaves during cooling. One plant was equipped with a cooling room containing washed air. Half of the 240 loaves involved were placed in this cooler before wrapping; the other half were cooled on racks on the platform in the loading shed. Total score representing extent of mold growth on the loaves from the cooler was 264. The appreciably greater mold growth as shown by a score of 508 was obtained on the bread cooled on the platform. Incidental tests in various plants established the fact that rate of mold growth was very generally directly proportional to the amount of exposure to dusty air which the bread had received.

Effect of plant sanitation.—General plant sanitation has a direct bearing on the amount of mold growth resulting from this exposure during cooling. One comparison was possible between two plants that differed somewhat in applied sanitary practice. A contributing cause in the mold development on the bread in one of these plants was the fact of rather free exposure of the bread during cooling to dusty air blowing in through open windows from the back yard. This exposure, combined with a possible reproduction of mold spores from growth in difficultly accessible places in the plant itself, resulted in a rather rapid development of mold on the products. This rate was decidedly greater than that on the bread from a neighboring bakery that was examined after a similar storage treatment. The latter plant had no mechanical cooler but was continually battling with mold spores everywhere on the premises and avoided dusty conditions during cooling of the bread as much as possible.

Effect of wrapping temperature.—Loaf temperatures at wrapping time were varied in several comparisons, accomplished by varying the cooling time. Temperatures of 135, 120, and 92 degrees F. were used.

Contrary to the expected, it was found that slightly less mold growth occurred on the bread cooled to 120 degrees than on that cooled to 92 degrees F. Both showed less mold development than the bread wrapped at 135 degrees F. The explanation is undoubtedly to be found in the increased exposure necessary to cool to the lowest temperature. If bread is not well protected during cooling, the increased number of spores falling on the loaves during the longer period may more than counterbalance the less favorable conditions for mold growth in such a loaf after wrapping. Probably the optimum wrapping temperature will vary with the degree of sanitation effected in the cooling.

Effect of baking time.—Variations in time of baking caused only small differences in rate and extent of mold growth. Tho the changes were in the expected direction, in that increased baking time reduced mold growth, the differences were smaller than might have been anticipated. However, none of the loaves were decidedly underbaked and if this extreme practice had been allowed, mold growth would probably have been much more abundant.

Effect of humidity in storage.—Extreme humidity during storage was again shown to be quite a factor in favoring mold development. This was noted by comparing the records obtained in the different plants in which climatic conditions varied the most widely during storage. It was also confirmed by the more rapid mold growth on bread sealed in cartons as compared to that on bread stored on shelves.

Effect of skimmilk solids.—The most conclusive result is obtained from the comparisons of milk-free bread with that containing 6 per cent dry skimmilk. Identical treatment was afforded these two types throughout the entire investigation. Scoring was carefully done and was in many cases the result of the combined judgment of several people. The differences observed in the rate of mold growth on these two types of bread were not great in any case. In certain series, one of these types might show a slight average advantage over the other while the next series might be reversed in order of rating. A total of 1,344 loaves, 672 of each type, was included in the comparisons. The average score for these loaves was 2,712 for milk bread and 2,756 for bread without milk solids. These figures show that the rate and extent of mold growth was slightly reduced by the use of 6 per cent dry skimmilk. There is probably not a sufficient difference to assume that dry skimmilk can be classed as an effective mold inhibitor. However, the results of these commercial tests and of laboratory experiments prove that if a baker is troubled with rapid mold growth on his product, he cannot help the situation and may aggravate it to some extent by reducing the milk solids content of the bread formula.

Increasing cleanliness of the air should be the aim of the baker in any battle against mold. Unless completely protected cooling is possible or unless sterilization at wrapping time can be accomplished, cleanliness can not be limited to the portion of the plant in which bread is cooled. All parts of the establishment must be continuously scrubbed and made more nearly sterile to reduce as much as possible the reproduction of mold spores. This requires sustained effort that is well repaid, not only in mold protection but in the increased pride felt by owners, employees, and the consuming public.

We cannot change the weather, so conditions will continue to be conducive to mold growth in certain seasons and areas. We may not be able to change greatly the resistance of our bread to mold growth by formula modifications, but a certain assurance may be gained from the fact that many bakers are successfully avoiding mold losses even in the most troublesome areas and are offering the public an attractive nutritive loaf of excellent keeping quality during the entire year.

SUMMARY

Baking kills all common mold spores that may be present in bread dough ingredients.

Dust particles carry mold spores to contaminate the surface of the loaf while cooling. Such spores will develop under suitable conditions.

Relative humidities of about 90 per cent are needed in the air surrounding the loaf to effect visible mold development within a seven-day period.

Bread containing 6 per cent skimmilk solids increases moisture retention after baking, thus retarding formation of moisture film between the bread crust and the wrapper. Milk-free bread releases its moisture more rapidly from crust to the air space within the wrapper.

Laboratory and commercial comparisons were made of the rate of mold growth on milk-free bread and on bread containing 6 per cent skimmilk solids using several baking, cooling, and storage procedures. In these commercial comparisons, humidity was again shown to be an important factor in the rate of mold growth.

Bakery sanitation was found to be of more importance in reducing mold troubles than thoroness of baking and cooling. The former is chiefly important in that it reduces the mold spore content of the air and thus the amount of loaf contamination.

Dry skimmilk caused an average lowered rate of mold growth on 672 pairs of loaves. Thus there is no justification for reducing the milk solids content of bread to prevent mold.