ON THE FORMATION OF "EYES" IN EMMENTAL CHEESE[1]

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INTRODUCTION

The quality of a prime Emmental cheese is determined not alone by its sweet, nutty flavor and pliant texture, but also by the character of its holes.

Cracks, which frequently mar the appearance of a cheese, are intimately connected with a faulty texture. So too are the holes dependent upon proper texture for their shape; but, while the holes have a more or less spherical form as if distended by a gas in a plastic medium, there are important distinctions superficially based upon their size.

The normal "eyes," familiar to every lover of "Swiss cheese," vary from the size of a hazel nut to that of an English walnut. When uniform in size and distribution they give "The King of Cheeses" a distinction admired by the connoisseur. To quote the words of an authority (24) on the manufacture:

> Beim Anbohren entschlüpft dem Kenner ein bewunderndes "Ah," wenn sich auf dem Böhrling zwei bis drei mattglänzende, sauber ausgearbeitete Augen von ein bis zwei Centimeter Durchmesser zeigen.

On the other hand there frequently occur large "blow holes" which not only may mar the contour of a cheese, but which sometimes are associated with an "off flavor." Last and worst are "die Nissler," "the thousand eyes," the "pin holes," which frequently ruin a cheese by making it spongy, and which always detract from its commercial value even when present in small number.

There is also a marked difference in the times at which the various classes of holes develop. So many apparent anomalies

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occur in cheese making that generalizations are dangerous, but, with a fair degree of truth, it may be said that "Nissler" holes develop while the cheese is under press or directly after; while normal eyes seldom start until the cheese is at least a week old. Again large holes seldom develop in press. They generally become evident later, and instances are noted by the European writers where they develop after normal eye formation.

Aside from the superficial distinction of size there are certain more fundamental differences of origin. Nissler holes undoubtedly have their origin in a gaseous fermentation of the sugar of fresh curd. Such a fermentation may be produced by bacteria or yeasts. When caused by bacteria the abnormal fermentation is revealed by the gaseous content of the holes, for it was shown by Clark (6) that the gas of Nissler holes may contain a large percentage of hydrogen, while that of normal eyes contains none. Various attempts have been made to distinguish the fermentations responsible for various types of holes. The greatest success has been met in the study of Nissler holes, for a number of bacteria and yeasts have been found whose gaseous fermentation of the sugar in fresh curd may clearly be held responsible. In like manner the formation of blow holes has been clearly traced in certain instances. The biological origin of normal eyes is still in doubt.

However, we are not now concerned with the bacterial origin of any of these openings, nor even with the texture of the cheese which is an essential prerequisite to their formation. The point to be discussed is merely the superficial distinction of size and its cause.

Granting that the distending gas in each case has its origin in a distinct fermentation, why should this superficial difference of size be so persistently characteristic? The time was when the different fermentations were far less clearly distinguished and the difference between pinholes, "eyes" and blow holes was considered to be merely one of size, and perhaps also of the period of cheese ripening in which they are formed. Baumann (2) made no other distinction than this and attributed the three general types of holes to the activities of the same organism.

Weigmann (33) adds:

> Auf dem Gebiete der Käsegärung haben wir gefunden, das die Käseblähung in den meisten Fällen von gewöhnlichen Milchbakterien bezüglich Pilzen ausgeht und die normale Augenbildung und die Käseblähung physiologisch dasselbe sind und sich nur quantitativ unterscheiden.

We now know the distinction to be qualitative, but whether it is quantitative or qualitative the same question may fairly be asked: Why is it that gas holes formed "in press" are generally small and numerous while eyes which develop slowly are large and comparatively few?

If a well based reason for this shall be found, we may have gained a better view of the more general aspects of hole formation, and a firmer grasp upon the means of scientific control we hope to attain.

Aside from its intrinsic interest the determination of the factors which influence the size and spacing of the eyes is of direct practical importance inasmuch as there are evidence that preference is turning gradually toward a cheese with larger and fewer eyes.

In 1896 Bächler (1) described the ideal Emmental cheese as one having eyes 1 to 1.2 cm. diameter, 2 to 4 cm. apart. Peter and Held (24), in the 1910 edition of their text book, give the diameter as 1 to 2 cm.; as does Konradi (19) who visited the Swiss factories in the summer of 1912. Thus between 1896 and 1912 the maximum size of an ideal eye increased from 1.2 cm. to 2 cm.

Certainly the cheeses which sell as imported over the counters of our local markets are characterized by eyes fewer and larger than those described by the European writers; while the "domestic Swiss" have more and smaller eyes. Whether the European makers select cheeses with larger eyes expressly to meet the demands of the export trade; or whether there is a differentiation between all cheeses of domestic or foreign make, which if texture and flavor are equal, brands a small eyed cheese as "domestic" and a large eyed as "imported," it is difficult to say. Nor is it essential to our purpose to discover, except insofar as the distinction in the market indicates a preference which the maker must meet.

In the following pages one very important factor in determining the size and spacing of holes will be presented.

THE RELATION BETWEEN BACTERIAL COLONIES AND EYES

It seems to have been assumed by many writers that, if bacterial action is the cause of the evolution of the gas, bacteria in sufficient numbers to produce this necessary gas must be strictly localized about a hole. This certainly is the most straightforward supposition to make; and, if true, it would seem as if a comparison of the flora about the eyes with the flora in other parts of the cheese would lead at once to the discovery of the organisms to which eye formation is due. Such comparisons have, however, not furnished the striking results we would expect.

That there does appear to be an unequal distribution of bacteria in hard cheeses is indicated by the investigations of several writers. Wigand (34) as early as 1884 stated that the bacteria in cheese are distributed in part as clusters. Inference of an unequal distribution was found by Duclaux (7) and by Troili-Petersson (31) in their observations that heavy inoculations from cheese sometimes gave no fermentation when smaller inoculations did.

Burri (5) describes a rare case in which dark colored colonies unassociated with "eyes" had become large enough to see.

Harrison and Connell (15) found a difference of 30 per cent in the bacterial content of different regions of Cheddar cheese.

In judging the value of the methods of study used in the investigations mentioned above it must not be forgotten that the transference of bacteria to artificial media for the purposes of counting and cultural tests is accompanied with the presentation to the bacteria of very different conditions from those found in cheese. None of the artificial media so far devised approximates exactly the relative great power of cheese to preserve a more or less constant hydrogen ion concentration with the consequent extension of growth and the control of enzyme action. Nor do artificial conditions always simulate cheese in furnishing the proper degree of anaerobiosis. Thus an organism or its liberated enzymes may be able to produce in cheese much more CO_2 than in artificial media; and, as the limits of growth and action are reduced by artificial media, large differences in gas producing power may become narrowed to such an extent that the powers of different organisms may appear the same. It may therefore be true that some of the organisms isolated from certain regions, although culturally appearing identical in number and kind to those isolated from other parts of the cheese, may indeed have far greater CO_2 producing power when growing in cheese.

Jensen (18), upon comparing the number of bacteria on the walls of eyes and in parts of cheese distant from eyes found no striking difference. In fact a glance at Jensen's table shows that often the preponderance was in favor of regions of the cheese distant from the eyes.

But let us see what evidence direct microscopical observations present. Upon the unequal distribution of the bacteria, as seen in cheese sections, Beijerinck (3), Maggiora, (22) Troili-Petersson (30), Harrison, (14) and Percival and Mason (23) agree.

Gorini (13) determined the distribution of bacteria in Grana cheese by sectioning both fresh samples and samples hardened with alcohol. He found bacteria in enormous numbers and their distribution he grouped under two classes. The first class was that of dissemination, that is, a more or less uniform distribution of uncolonized organisms. In the second class were grouped the large colonies similar to those of plate cultures.

Rodella (25) supplemented the ordinary methods of histology by pressing cubes of cheese between two glass slides and staining the cheese which adhered to the glasses when they were withdrawn. He came to much the same conclusion regarding the distribution of bacteria.

The unequal distribution, as Gorini pointed out, should make us skeptical of "counts" as ordinarily made; not only because of the difficulty of obtaining a homogeneous sample with such an imperfectly soluble substance as cheese, but because the bacteria are so unevenly distributed. This should add to the value of the direct microscopical methods, although Boekhout and Ott de Vries (4) have protested that the scattered bacteria found in sections may have been smeared off the colonies by the knife, and Troili-Petersson suspects that many of those seen are the borders of colonies on other sections. On the other hand Boekhout and Ott de Vries claim, that many of the colonies they observed in Edam sections were of dead bacteria, and consequently were not ripening centers. To this Löhnis (20) replies that ripening may not depend upon the living bacteria but upon their liberated enzymes. Whether such enzymes are able to diffuse from the centers where they are produced may be open to question; although from analogy with the ripening of soft cheese, where it is certain that the enzymes of surface moulds penetrate slowly toward the center, we must assume that they do.

The weighed evidence of microscopical examination supported as it is by Rodella's supplementary method, seems to indicate rather clearly that the bacteria are grouped in large clusters.

But the point in question is whether these colonies are correlated with the holes. Troili-Petersson (30) found lying in the walls of the holes of Swedish "Güterkäse" long slender colonies of bacteria spread out parallel with the walls. This vegetation, however, appears not to be of any one species of bacteria, and the form and size of the colonies may have been simply due to their having the space in which to spread. Beijerinck (3) who made sections of Edam cheese expresses the opinion that the accumulations of colonies of bacteria and crystals of tyrosin, etc., which he observed are due to local causes but he speaks of no relation between colonies of bacteria and the gas bubbles he observed. Maggiora's (22) description of sections of overripe cheese give no further information. Rodella was more concerned with the relation of bacteria to ripening than with localization, and furnishes little information upon the point we are interested in. But Gorini (13) mentions in particular that no constant relation between these accumulations and the small cracks and holes of Grana cheese was observed.

It therefore appears that direct microscopical examination has afforded very little evidence to support the view that eyes develop where colony growth is greatest. In fact it is perhaps not pedantic to say, that, even if these methods had enabled us to observe strikingly greater aggregations of bacteria about holes they would afford no conclusive evidence on the point at issue since they do not take into consideration the physiological powers of the organisms. This leaves the results of microscopical examination open to the same objections previously made against the cultural studies so far used.

Clark (6) has shown that not only regions about eyes but solid parts of cheese distant from eyes are active in the production of gas. In the experiment described the eye regions were, indeed, the more active; but in another experiment conducted since the publication of the first, no difference was observed. These observations, combined with the fact that the solid cheese mass itself contains very considerable quantities of CO_2, invalidate any such calculation as that made by Jensen (18) in one of his earlier papers in which he attempted to show that the gas produced by Freudenreich's *Bacillus E* was sufficient in that it furnished somewhat more than enough gas to fill the "eye" space of an ideal cheese.

Until the specific origin of the gas is more definitely known, and until these bacteria have been located in greatest abundance at points of eye formation, or their liberated enzymes have been shown to have their action confined to these localities, the evidence at hand is in favor of the view that the gas is produced more or less evenly throughout the whole body of the cheese.

One further argument is almost sufficient of itself. If eyes start about colonies, how is it that these colonies are so sparsely distributed? In a prime cheese of 1896 the eyes according to Bächler (1) were rather evenly spaced 2–4 centimeters apart. With the development of the modern large-eyed export cheeses, the spacing of the eyes has increased greatly. *Yet colonies of bacteria occur so thickly distributed that they may be seen in almost any microscopic section.*

THE FORMATION OF GASEOUS AGGREGATES

There is really little reason, as well as little evidence, to support the assumption that the gas *necessarily* separates as gas bubbles where it is produced. It is not at all irrational to suppose that the gas, having first saturated the cheese mass, separates at advantageous points which have no *necessary* relation to those localities rich in bacterial growth. In other words we may suppose a process similar to the growth of crystals to take place.

Everyone is familiar with the principal phenomena of crystallization, with the fact that to start crystallization from supersaturated solutions it is often necessary to "seed" them, and with the fact that the slower the rate of separation the larger are the crystals obtained.

A step nearer the point we are concerned with is found in the case of rain formation. Lord Kelvin (28) showed that the vapor pressure over a curved surface differs from that of a plane surface. If the curvature is convex the vapor pressure is greater than that of a plane surface. Neglecting the very important factor of electrically charged nuclei, raindrops must therefore form first upon some object such as a dust particle which presents a surface more nearly plane than that of a minute droplet. Were a minute droplet to be formed in an atmosphere just saturated with its vapor, the curvature of its surface, and consequently its vapor pressure, would be so large that it would immediately evaporate while condensation were still taking place on larger drops and dust particles. Thus we may say large drops are formed at the expense of small ones.

Attention should be called to the fact that the alteration in vapor pressure is exceedingly small until the drop becomes exceedingly small, with a diameter of perhaps a millionth of a centimeter; nevertheless the difference is sufficient to prevent the precipitation of innumerable minute droplets from the atmosphere and to determine the growth to a larger size of drops already formed.

With only slight modification Lord Kelvin's treatment of the vapor pressure at curved surfaces may be applied to the gas pressure at curved surfaces of a gas in solution.

Using Lord Kelvin's diagram, figure 1, let it represent a closed space containing an aqueous solution of carbon dioxide with plane surface at *B*, a capillary tube in which this solution rises to the point *A* where it retains a concave meniscus. Let the remainder of the space contain only gaseous CO_2 and water-vapor.

Let us assume that the density σ of the carbon dioxid remains uniform throughout the height h, and that it is acted upon by gravity with the constant acceleration g. Let the pressure of the carbon dioxid in its liquid phase be w at the plane surface *B*, while at the curved surface *A* it is w'. Then w' must be less than w by $g\sigma h$. Were it not so, CO_2 would distill from *A* and condense at *B* and work could be obtained contrary to the laws of thermodynamics.

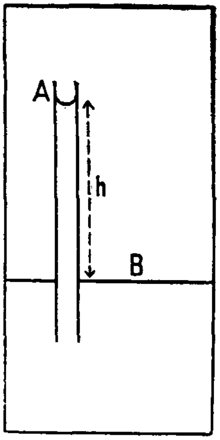

Fig. 1

Since vapor pressure and surface tension are related it may be profitable to look at this matter in another way. The pressure P tending to keep a bubble of gas spherical when suspended in a liquid is $P=\frac{2S}{a}$ (Willows and Hatschek) (32) in which S is the surface tension of the surrounding film and a the radius of the bubble. Since P balances the surface tension the pressure of the inclosed gas must overcome the surface tension if the bubble is to expand, but since P is inversely proportional to the radius of the bubble, it is more difficult for very small bubbles to grow than for large bubbles.

If the separating gas already finds a film of gas, it will separate there rather than overcome the enormous force necessary to form *de novo* a tiny bubble.

This explains the observations frequently noted that any body having an adhering film of gas becomes covered with bubbles when placed in a solution saturated with gas.

There is then a striking analogy between the growth of crystals, the formation of rain drops and the growth of gas bubbles in a solution; an analogy whose physical manifestations are numerous and whose theoretical basis has long been accepted.

The quantitative estimation of the relationships established has been purposely avoided in the above treatment, because it would be difficult to apply them to such a heterogeneous substance as cheese or even to colloidal gels such as those of agar or gelatine. Nevertheless there is no reason to suppose that the principles do not apply to such gels; and by using these viscous media, which are capable of retaining gas bubbles as water solutions can not, we may obtain some striking verifications.

THE SEPARATION OF GAS AT POINTS DISTANT FROM THE SOURCE

If a sterile nutrient sugar solution of agar or gelatin be sown while molten with a pure culture of some gas-producing bacillus, such as B. coli, and then allowed to set, the gas liberated after a period of incubation separates as bubbles which are held in suspension. It can then be clearly seen that numerous colonies of bacteria have developed at some distance from the gas bubbles. The logical conclusion must be that the gas after having saturated the gel does not necessarily separate where formed, but tends to diffuse and separate into a bubble already started at some advantageous point. A more striking example is to be seen in the following experiment:

A hot sterile non-nutrient agar solution was poured into a sterile test tube, and when sufficiently viscous to retain a bubble of gas in suspension, such a bubble was introduced by blowing through a sterile cotton-plugged glass capillary. To seal up any channel left by withdrawing the capillary a layer of hot sterile agar was poured upon the first. When this was thoroughly set an emulsion of nutrient agar and *B. coli* was poured on top. It was found that after a period of incubation, when the bacteria were presumably producing gas very vigorously, the pre-formed bubble in the non-nutrient agar increased very markedly in size. This experiment was repeated several times' with uniform success. It may justly be taken to illustrate the hypothesis that the gas in a saturated colloidal gel will obey the principle deduced for pure aqueous solutions, namely, that large bubbles will be formed at the expense of small.

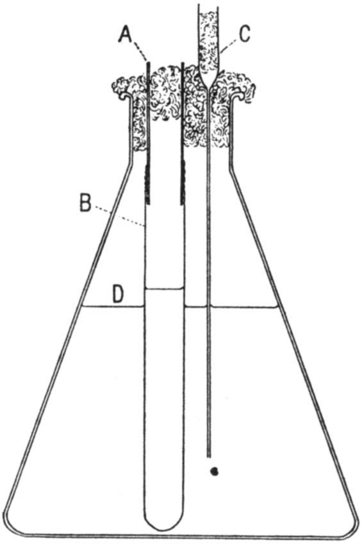

FIG. 2

In the above experiment it might be claimed with justice that into the original agar jelly there diffused sufficient food material for the bacteria to produce gas there; but it is improbable that within a few hours the bacteria in the supernatant culture could grow into, or by any probable means find their way into, the region where the initial bubble was blown. Indeed no growth about the bubble was observed.

In order to definitely preclude this source of error, the following arrangement was made (see fig. 2):

A collodion sac *B* was cemented with collodion to the glass tube *A*. It was then suspended in an Erlenmeyer flask. A nutrient sugar medium containing 1 per cent agar filled the Erlenmeyer within and without the sac to the level *D*, this level being considerably below the point where the sac was cemented to the glass tube. A very fine capillary tube *C*. with its upper end tightly plugged with cotton was then placed as illustrated, and the neck of the flask tightly plugged with cotton. The whole apparatus was then sterilized in an autoclave.

While the agar was still molten and at a temperature of about 40 degrees, that in the sac was inoculated with *B. coli;* and, when the agar on the outside of the sac was sufficiently viscous to hold gas bubbles in suspension, a few were blown in it by means of the capillary tube *C*. This capillary was then withdrawn. When all the agar had set, this flask was left at room temperature (about 22°) to incubate the bacteria.

As in the former experiment the pre-formed bubbles grew in size. The collodion sac was found to be intact at the close of the experiment. It had purposely been made rather thick to prevent the bacteria making their way through it. The above experiment was made in duplicate with like result.

Although Todd (29) found that collodion membranes were not impervious to B. *coli*, *Fuller* (11) the next year (1910) obtained results entirely at variance with those reported by Todd. Using Frost's (10) method he found it possible to make sacs which retained their bacterial integrity for several months. The same conclusion seems to have been reached by Heymans (17) (1912).

Although the experiments of Todd and Fuller contradict each other, it should be remembered that both investigators used fluid media while in the above experiment the medium contained 1 per cent agar. The collodion thus served simply as an additional barrier. Frost using *B. typhosus* and *B. pyocyaneus*, two organisms which Todd claimed penetrated collodion sacs, found that when the sac was embedded in gelatin, the organisms were retained perfectly.

There is then, every reason to believe that the bubbles which grew outside the sac and which were indeed more than 2 cm. from the sac were thoroughly separated from any bacterial contamination.

But if the proposed explanation be true, there follows an almost necessary corollary. To induce growth of a crystal time must be allowed for delicate adjustment of equilibrium else new crystals will be formed soon to compete for the substance in solution upon an equal basis with the first formed crystals. In like manner the rate of gas production must be low if a bubble already formed is to grow without competition, for if the gas is formed rapidly it can not become diffused and distributed from the points of production rapidly enough to prevent supersaturation of these regions. When this supersaturation becomes sufficiently great the gas must separate from solution. Consequently when the rate of gas production in a culture is rapid many small bubbles will be formed; and when the rate is slow the tendency will be toward the formation of larger aggregations or larger bubbles.

An illustration of this is furnished by Mr. Ayers of this laboratory. In some studies on the Wisconsin curd test Mr. Ayers found that curds containing numerous gas producing bacteria, and consequently subjected in general to a high rate of gas production, were filled with numerous very small holes, while curds containing fewer bacteria, and consequently subjected to a lower rate of gas production, were inflated with larger holes. It may of course be said that the number of holes correspond to the number of colonies; not a numerical correspondence, for such does not occur, but a parallelism. On this basis we might perhaps explain Mr. Ayers' observations by saying, that when a certain number of colonies in a thickly seeded culture have formed a small bubble, other groups of colonies throughout the medium have done likewise and at the same time have formed sufficient acid to prevent further growth of bacteria and consequently further gas formation. The result would then be numerous small bubbles. On the other hand if the colonies are very much less numerous the surrounding regions would furnish by diffusion both more food per colony and more absorption of acid per colony. In consequence, large holes would be produced. This explanation while plausible is only partially justified. In the first place there is no numerical correspondence between the number of colonies and the number of holes as can be seen with the naked eye in a clear gelatine culture of *B. coli.* It must therefore be granted that the gas, before it separates, actually does diffuse from the points where it is formed. Postulation of extra-cellular gas-producing enzymes would only emphasize this, since such enzymes if at all diffusible would be even more widely distributed than the bacteria. But in the second place, the writer has found that of two 200 cc. flasks of the same gelatine media each inoculated with 760,000,000 bacteria, that kept at 15° had larger gas holes than that kept at 20°. It is difficult to explain this except on the basis that the 15° culture had a lower rate of gas production which allowed time for larger bubbles to grow just as in the crystallization of salts the larger crystals will be formed during the slower crystallization.

Although factors other than the rate of gas production may influence the size and number of gas holes in a culture, the chief factor seems to be the rate.

APPLICATION OF THE PRINCIPLE TO CHEESE

It therefore follows that a rapid production of gas in cheese would result in the formation of numerous small holes, while with a slow rate the holes would tend to be large. In general it may be said at once that such a relationship does occur. The Nissler holes of Swiss cheese are small, and they are formed rapidly. The eyes are only formed after some time has elapsed, and grow with extreme slowness.

At this point it may be well to comment upon some objections which have doubtless occurred to the reader. In the first place are there not in cheese a sufficient number of gas bubbles enclosed in the curd during the manufacture to furnish innumerable gaseous nuclei for the separation of innumerable bubbles instead of relatively few "eyes." Examination of curd grains in the kettle do indeed sometimes show that they have adhering to them bits of froth, but it must be remembered that when the cheese goes to press its temperature is high and that these tiny bubbles may be absorbed when the cheese cools. If one blows *tiny* bubbles in a viscous solution of agar (40°) or in a fairly warm gelatine solution, these bubbles will be seen to entirely disappear as the solution cools—provided of course the bubbles were originally not too large. There can be little doubt that a like absorption takes place in cheese.

It may further be questioned whether the solid particles of cheese do not furnish innumerable nuclei for the separation of gas bubbles just as dust in the atmosphere furnishes the nuclei for the condensation of rain. To this question we have a positive answer in the experiments of Gernez (12).

Gernez found that in the separation of gas from supersaturated solutions solids alone do not serve as nuclei. A film of gas upon the solid surface is an absolute essential. If a glass rod is plunged into a supersaturated gas solution bubbles are formed upon those surfaces which had been exposed to the air; but if the rod is broken while in the solution no bubbles form upon the freshly exposed surface. Likewise, precipitates if formed in gas-free solutions, do not serve as nuclei for bubbles when placed in supersaturated gas solutions. It appears then that a solid, if it is to serve as a nucleus for a gas bubble, must have a surface film of gas.

We are therefore justified in believing that the cheese curd goes to press with few so-called nuclei of any kind which may induce the growth of gas holes.

But let us see what further evidence there is that the formation of gas holes in cheese does follow the analogy to crystal growth which has been proposed.

Freudenreich (8) in his cheese making with *B Schafferi* found that this organism could produce Nissler cheeses and also blown cheeses; and he therefore considered that these faults are not necessarily due to different bacteria.

Freudenreich's explanation was as follows: If the bacteria are allowed to develop to such an extent before the cheese is made that they are numerously and evenly distributed throughout the milk, Nissler holes are formed because there are numerous colonies. If, however, the cheese is made up directly after inoculation the colonies are fewer and the cheese develops blow holes. Undoubtedly there is a good deal in this theory; but it should be asked if even in Nissler cheeses we are to assume the number of colonies equal only to the number of gas holes, and especially so when these holes are several centimeters apart. If we look over carefully Freudenreich's paper we shall find in the first place that his Nissler cheeses were those which gased in press while his blown cheeses took a week or ten days to blow; indicating that the time factor was an important one, and that what we may call the crystallization of the gas was the important factor. Furthermore it is significant that in at least one of Freudenreich's crucial experiments the bacteria introduced were not allowed to develop before but *after* the addition of the rennet. Now it is known that rennet acting upon pasteurized milk such as Freudenreich used in this experiment takes longer to produce a firm enough curd for cheese making; nevertheless it rapidly, sometimes more rapidly than in raw milk, produces a thin coagulum. Therefore in Freudenreich's experiment we would surely not expect the bacteria to have been scattered after the addition of the rennet but to have attained larger colony growths. If this assumption be correct we would have expected a blown cheese according to Freudenreich's hypothesis. Instead he obtained a Nissler. The simpler explanation appears to be that he obtained in this case colony growth of such extent that a rapid gas production took place, and that, in consequence of this high rate, the gas had to separate close to the points where it was produced.

Of course this interpretation must not be construed too rigidly. If there are present in the milk particles of cow dung, the infection of the cheese may become so rank at certain points that nothing short of a blow hole will be produced at these points. Jensen (18) actually observed this correlation and Freudenreich (8) found that paper pellets soaked in a culture of *B. Schafferi* produced blow holes in the cheese about these rank infections.

In such a case the gas, though it may separate at frequent points near its origin and though it may tend to produce a "Nissler" cheese can not stop short of the production of a "blown" cheese with large holes because of the abundance of the gas which must separate. The holes of such *rapidly* blown cheeses however reveal the manner of their formation by the irregularity of their contour. They appear to have been distended by a more or less explosive gas production and are without that clean-cut, neatly spherical contour of the perfect eye, which results when time is allowed for the adjustment of tensions.

STAINED CHEESES

One test to which we may subject the hypothesis which has been suggested is the following: If Nissler holes are formed at the points where the gas is produced or even close to those points, then no particular locality in the cheese should be favored provided the bacteria are distributed both within and without the curd grains. On the other hand, if normal eyes are formed so slowly that time is given for the gas to assemble and separate at favorable localities, we should expect to find these localities to be of some definite nature.

That the bacteria in fresh curd are distributed both within and without the curd grains can not be doubted, although it may be that their numerical distribution differs. Harrison and Connell (15) state that upon inoculating milk with a gas-producing organism more of these were found on the exterior of the curd particles than within. Russell and Weinzirl (26) found fewer organisms in the curd than in the expressed whey.

On the other hand Hastings, Evans and Hart (16) find that the curd retains the greater part of the bacteria found in milk. These observations apply to Cheddar curd. Freudenreich and Jensen (9) observed that in the manufacture of Swiss cheese the greater part of the bacteria were to be found within the curd grains.

If it be permissible to draw a definite conclusion from this, it is that the method of manufacture of Swiss cheese, and especially the high cooking temperature, is least destructive to the bacteria within the curd. We should therefore expect the gas producers in a Nissler cheese to be distributed both within and without but predominatingly within the curd grains.

In 1896, Bächler (1), an experienced cheese maker, proposed that eyes are formed between curd particles. The writer has been unable to obtain a copy of Bächler's original paper but from abstracts has gathered that his view was as follows:

When the curd is hooped, if the whey is not thoroughly expelled from between the curd particles, pockets of whey will be retained. After the cheese leaves the press the whey from these pockets will be absorbed and a "weak" spot left. Whether Bächler meant an actual hole or a place where the curd grains were imperfectly matted is not clear. The latter interpretation is probably more just, for Bächler must have observed the irregular holes, so-called "mechanical holes," which sometimes occur in weakly pressed curd, and he would not have mistaken these for incipient eyes. A normal eye from the moment of its origin retains its characteristic spherical shape, and, when small, closely resembles a Nissler hole. It is probably imperfectly matted curd grains which Bächler meant by weak spots.

If this be so, it is obvious that a method of testing his hypothesis would be to so stain the surface of each individual curd particle that in the solid cheese its outline would remain distinct. If in a cheese so stained a gas hole should form *between* curd particles its interior wall would be *colored*; while if it should originate *within* a curd grain the interior wall would remain *uncolored.* An admirable dye for this purpose was found in Congo red. When this was sprinkled into the kettle just before the curd was drawn it stained the surface of each curd grain a uniform red and did not penetrate. A cross section of a cheese so stained revealed the distinct outline of each original curd particle.

Two stained Nissler cheeses were made by the addition of cow dung to the milk. Upon cutting these cheeses when taken from the press it was found that the gas bubbles were not correlated with any particular locality. In numerous instances they were clearly seen to be wholly within the curd particles, while in other cases they had pushed aside the walls and formed holes whose interior walls were stained.

On the other hand, stained and apparently normal cheeses which developed apparently normal eyes presented a very different appearance when cut. Almost without exception the holes had the characteristic appearance of normal eyes, and *their interior walls were all without exception stained.*

There can be little doubt therefore that no particular locality is favored when Nissler holes form while, at least in the experimental cheeses, the eyes without exception developed between curd grains. This is in harmony with the hypothesis that the gas of Nissler holes, because it is formed rapidly, must escape from solution near where it is formed, while the gas of eyes, having time to diffuse and to keep a closer equilibrium between its gaseous and liquid phases, separates first at a favorable locality, and there forms an aggregate comparable with a crystal.

We must be careful to say that "at least in the experimental cheeses the eyes developed between curd grains." The dye used injured to a slight extent the matting quality of the curd, so that there may have been produced artificially those weak spots suggested by Bächler. If so, it alters in no way the validity of the argument that eye development takes place in favored localities, although some slight doubt may be thrown upon the conclusion that in an undyed cheese these places are between curd grains.

Closer examination of the eyes of dyed cheese reveals an interesting point. It was noticed that in a great majority of cases the interior walls of the eyes were not uniformly stained. A small portion was unstained and almost of as clear a white as an undyed curd grain. Inspection of the curd grains in the vat showed that they were generally uniformly stained but occasionally enfolded surfaces and occasional unopened cracks were detected whose surfaces the dye had not reached. An eye developing in contact with such a surface would have its interior wall only partially colored.

In regard to the nature of the localities at which the gas separates there is, beside the hypothesis of Bächler (1) that of Schaffer (27). Schaffer's studies of eye development as followed with the X rays led him to believe that the regions of eye growth were regions of sufficiently active proteolysis to allow the curd to be absorbed and give way to the expansion of the gas. Schaffer tried to explain away Jensen's finding that there was no distinct difference in the composition of the cheese near eyes and distant from them; but, while we admit that Jensen's method of analysis was not very delicate, we must also admit that Schaffer's evidence is of somewhat dubious value.

Why any particular locality should be favorable to the development of a gas bubble it is difficult to say; but from the striking appearance of the eyes of cheeses stained with Congo red, it is very evident that the eyes *do* start at particular points. Whether or not the eyes originated between curd grains, the surfaces of the eyes ultimately involved the surfaces of the curd grains.

Perhaps the unequal coloring of the eye surfaces is due as suggested to an unstained surface being involved; perhaps it is due to some proteolytic effect such as Schaffer has suggested whereby the interior of the curd grains became exposed; perhaps the Congo red was reduced.

Whatever the situation may be, the fact remains that the stained cheeses clearly demonstrate a difference in the locality of Nissler holes and normal eyes, a difference which demonstrates the only point with which we are now concerned; namely, that a sudden evolution of gas will result in many small gas bubbles located where the gas is produced, that with a slow evolution of gas, separation takes place at favorable places only.

The position of any particular eye, its rate of growth and its ultimate size depend upon a great many factors. We have not considered the influence of the texture of the cheese, its permeability to gas, the tension of the rind, nor the influence of proteolysis, and of the fat content upon the surface tension of an eye surface. These all must be considered in time, and to prevent confusion each should receive attention separately. All of these factors however are merely modifying influences, and none affects the validity of the argument that the gas in separating into bubbles in cheese follows the same laws that it does in beer and the same laws that apply in different degree only in the separation of gaseous liquid or solid aggregates from their saturated solutions.

SUMMARY

A review of the literature reveals little or no evidence that the eyes of Emmental cheese are strictly localized at points of excessive bacterial growth. On the contrary the evidence of bacterial counts, and direct microscopical examination as well as the gas production of different regions of the cheese indicate a more or less uniform distribution of the eye distending gas.

Certain theoretical considerations are presented which lead to the hypothesis that the gas separates in aggregates according to laws governing the separation of gas from supersaturated aqueous solutions. This hypothesis has been tested upon viscous media with results directly applicable to the "eye" and "Nissler" hole formation in cheese.

It is concluded that the gas produced in Emmental cheese separates in aggregates whose localities have no necessary relation to the points where the gas is produced, that a rapid gas production must tend to the formation of numerous small holes while a slow gas production must admit the formation of larger holes. This conclusion is shown to agree with the fact that Nissler holes are produced by a rapid fermentation while eyes are formed slowly. This conclusion also suggests that the gas of Nissler holes must separate at numerous points near its point of origin without regard to any particular locality of the cheese; while the eyes must form at favorable points.

This was experimentally verified by a study of stained cheeses.

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