BACTERIAL SPOILAGE OF A THOUSAND ISLAND DRESSING

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INTRODUCTION

The commercial preparation of mayonnaise and similar dressings is one of the newer industries which has arisen from a greater knowledge of nutrition. With the discovery of vitamines in green vegetables and the subsequent increased consumption of lettuce, celery, cabbage, asparagus and other vegetables, came the need of a dressing to make them more palatable. The French originated this sauce and it had been prepared in the home with more or less success. Preservation of the product has not been an important factor in the home-prepared dressings since they are ordinarily used shortly after preparation. The lack of knowledge of the value of egg yolk in stabilizing the emulsion and of vinegar in preservation, the use of proper amounts of seasoning and the proper ratio of the various ingredients has proved a serious handicap to the commercial development of this industry, but in spite of this, production has increased by leaps and bounds.

Until recently the problem of stabilizing the emulsion attracted more attention than the problem of preservation. Since dressings of this nature are altered by heating, they cannot be preserved by sterilization and other methods must be practiced. Further complications ensue when mayonnaise is mixed with materials such as chili sauce, pickles, olives, pimentos or ground meat in the preparation of Thousand Island dressings, sandwich spreads and similar materials. The original preservative material may have been of sufficient concentration to inhibit the growth of

bacteria, but for example, when diluted with chili sauce, growth of the bacteria present in the mayonnaise may follow. Such a condition was called to our attention recently and it was the purpose of this work to discover the cause, note the effect and eliminate the spoilage.

HISTORICAL

The separation of oil and occasional subsequent fermentation has been noted since the industry began. Iszard (1927) examined about fifty samples and noted three stages in the spoilage. Gas bubbles appeared first, followed by a rancid odor of butyric acid and finally by a separation. After separation organisms sometimes develop. The majority of these samples were fermented by the aerobic spore formers *B. petasites* Gottheil or *B. teres* Neide. The organisms could be isolated from the foundation paste but since this could not be sterilized, she studied the comparative value of lactic acid and vinegar as preservatives.

Bachmann (1927) studied a number of commercially prepared dressings finding spore bearing rods similar to *B. subtilis* Cohn and *B. mesentericus* Trevisan, as well as molds, micrococci and diplococci but no toxin producing anaerobes. In no case were all of the samples from any one manufacturer sterile. A close relationship was found between the number of organisms present and the acidity and added starch paste. The eggs were considered to be the source of the organisms.

Rettger (1914), Hadley and Caldwell (1916) and others have found that the majority of fresh eggs are sterile, but aerobic spore formers have been noted.

Gray (1927) found in pimentos a spore former which was heat-resistant to a considerable degree. He suggested sterilization of the separate ingredients, especially those added to mayonnaise to make sauces.

Bitting (1928) in studying the stability of the emulsion, noted that as the proportions of the ingredients approached the stable point, the loss due to growth of organisms was lessened.

THE FERMENTED DRESSING

The spoiled dressing which was studied was composed of a mayonnaise base made from corn oil, vinegar, egg yolk, sugar, paprika, mustard and pepper to which was added pickles, olives and chili sauce. The product was ordinarily a smooth, heavy-bodied emulsion of a light orange color. The acidity as determined by titrating with N/10 NaOH using phenolphthalein as an indicator was 0.71 per cent as acetic acid. The hydrogen ion concentration was between pH 4.2 and 4.4.

The spoiled material was darker in color and showed separation of oil in varying degree. The oil could not be incorporated satisfactorily by beating. The titratable acidity had increased, one sample titrating 0.91 per cent acid. The hydrogen ion concentration had also increased to pH 4.0 to 4.2. The volatile acidity had increased from 0.55 per cent in the normal to 0.60 to 0.64 in the spoiled dressing.

Microscopic examination showed the presence of a large number of spores in all the samples of spoiled material examined. Apparently only one species was present. Transfers made into yeast extract agar slants showed abundant growth in twenty-four hours but no spores until the second day. Several of the samples were plated, isolations made, and the organism studied in detail. Only one type of colony developed upon the plates.

CHARACTER OF THE CAUSATIVE ORGANISMS

The morphological and cultural characters are as follows:

Morphology. The organisms are from 0.5 to 0.8 microns by 1.5 to 3.0 microns long on glucose agar. Chains up to 10 microns in length have been noted in very young cultures, but ordinarily organisms occur singly. On potato slants and in gelatin stab, the organisms are somewhat larger. They are actively motile.

Staining properties. They are Gram-positive.

Spore formation. Spore formation begins after twenty-four hours on glucose agar. Spores are usually central, but occasionally eccentric. Spores measure from 0.5 to 0.6 microns by 0.7 to 1.0 microns. The protoplasm at the ends when attached may

shrink giving the appearance of a bulging cell. The protoplasm ordinarily remains attached to the spore.

Growth on agar slant. Growth on agar slants is abundant, heavy white and wrinkled. During the first twenty-four hours the growth appears moist and glistening, but later it becomes dry and dull. The growth is membranous and lifts easily from the agar. A decided odor is noted.

Growth in agar stab. The growth is heavy and wrinkled on and near the surface, but very faint further down along the line of inoculation.

Agar colonies. Colonies after two days are spreading, white, ameboid with edges entire. The surface is finely granular.

Growth in broth. A pellicle forms on broth, the liquid becoming slightly cloudy.

Growth in gelatin stab. Growth is rapid, and liquefaction complete in two days at 18°C.

Growth in litmus glucose agar stab. On litmus glucose agar stab a heavy surface growth appears with a faint growth along the line of inoculation. The medium becomes alkaline with a slight reduction at the bottom. Later the medium becomes alkaline from the top toward the bottom.

Growth on potato. Growth on potato is profuse, spreading and slimy with a heavily wrinkled surface. Growth is grayish, and the potato is discolored to a brown or pink.

Indole production. Negative.

Reduction of nitrates. Nitrates are reduced on peptone or synthetic agar slants.

Growth in litmus milk. The milk is peptonized completely. The color becomes purplish.

Growth in glucose broth in fermentation tubes. The growth is luxuriant with a pellicle in the open arm. No gas is produced.

Growth in carbodydrates and related compounds. Acid is produced in glucose, fructose, lactose, maltose, sucrose, raffinose, rhamnose, inulin, dextrin, starch, glycerol, mannitol, sorbitol and salicin, but not in xylose. The hydrogen ion concentration attained is from pH 5.6 to 6.0.

This description is similar to that of *Bacillus vulgatus* Trevisan as given by Bergey (1930) or by Ford (1927).

THE SOURCE OF CONTAMINATING ORGANISMS

When this problem was called to our attention it was believed that the chili sauce contained the contaminating organism. This was the natural conclusion, since the spoilage did not occur when the chili sauce was omitted in the preparation of dressings. With this in mind, microscopic preparations were made and examined, the chili sauce was plated on various types of agar, and inoculated into various broths, but no sign of living organisms could be noted. Samples were then obtained of the other ingredients of the dressing.

Since Bachmann (1927) believed the eggs to be the source of contamination and Rettger (1914) showed the presence of aerobic spore formers in eggs, the frozen egg yolk was next examined. The product appeared to be normal in every respect. Microscopic examination showed the presence of non-spore forming, Gram-negative rods, but no spores or Gram-positive rods. Knowing that if the spore formers were present they would be there in small numbers an attempt was made to kill off the non-spore forming bacteria and then by plating to recover the spore formers. With this in view 5 grams of the egg yolk were diluted with 100 cc. of water heated for thirty minutes at 150°F. and then plated. The colonies found developing upon these plates were few in number and of one type. Microscopic examination showed the organisms to be Gram-negative, short rods. The organisms produced abundant acid and gas in carbohydrate media, reduced nitrates and formed indole. They failed to form spores. If any spores were present in the egg yolk, the characteristic colonies should have formed on the plates, but none of the colonies showed any resemblance to the colonies ordinarily formed by true bacilli.

It did not seem to be possible that the spices could be the source of the contamination, since Bachmann (1916) showed that spices have some preservative value especially in concentrated form. Cutter (1922) found *Cl. botulinum* Holland growing in an acid tomato onion sauce. Bachmann (1923) found the same organism in a highly spiced mince meat and Albus and Ayers (1928) found that pimentos may have been the cause of gassy fermentation in

processed cheese. With these facts in mind a thorough study of all the ingredients was made.

Approximately 0.1 gram of pepper, mustard, paprika, salt and sugar and 1 cc. of vinegar were plated directly using a peptone glucose agar. The three latter materials failed to produce colonies in two days at 25°C., but colonies developed on the plates from pepper, paprika and mustard. Only two colonies developed on the mustard plate, one a colon-type colony, the other similar in every respect to those from the dressing. The number of these colonies was not great, but since they spread so quickly, the plates were practically covered. Organisms were isolated from these plates. Comparative studies showed that the bacteria in the pepper and the paprika were identical with those previously isolated from the dressing. The organisms from the mustard showed but slight differences which suggests that they were a closely related species.

STUDY OF OTHER SEPARATED DRESSINGS

Other samples of separated mayonnaise, Thousand Island dressing, sandwich-spread and Russian dressing were studied in a similar manner. With the exception of a sandwich-spread, none of these materials showed an appreciable number of organisms and even after standing several months at room temperature no further change was noted. The separation was therefore believed to be due to an unstable emulsion.

The sandwich-spread showed a number of spore formers present. Morphological and cultural studies of this organism showed it to be similar to, but not identical with, those previously described. Not all the ingredients were available for study in this case and therefore it was impossible to make a more complete study. Of those materials available the corn starch was the only one which contained spores of bacteria.

METHODS OF PREVENTING LOSSES

Since it is impossible to sterilize the final product, some other method of preventing losses becomes necessary. Two methods of

attacking this problem are possible. In one, the acidity of the product may be increased so that organisms can not grow. This may be accomplished by the use of lactic acid as shown by Iszard (1927). It may also be accomplished by the addition of more vinegar, but difficulties may arise in that the emulsion may be unstable if too much vinegar is added as shown in the work of Bitting (1928). In certain cases, an acidity high enough to inhibit the growth of such organisms as these spore formers is undesirable.

A second method of attack is to kill the organisms before the contaminating material is added to the product. In this case this would be the pepper and paprika, and possibly the mustard.

A test was carried out in which the pepper, the paprika and the mustard were placed in vinegar, allowed to stand five minutes and then plated. The vinegar was then brought to a boil, the boiling continued for five minutes, samples being taken for plating at one minute intervals. Samples boiled for one minute showed a decided reduction in number of organisms and after three minutes no growth appeared in any of the samples. This method of sterilization could, of course, be applied to certain of the ingredients of the dressing only.

CONCLUSION

An outbreak of spoilage of Thousand Island dressing was found to be caused by an aerobic spore former, *Bacillus vulgatus* Trevisan.

The contaminating organisms were found to come from the pepper and the paprika used in the dressing.

Methods of sterilization may be of two types: (*a*) preparing a product of such acidity that it will not allow the growth of organisms, or (*b*) sterilization of the ingredient which contains the contaminating organisms.

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