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Seventeen years of study in North Carolina have yielded 40 contributions to the art of pickle processing. By JOHN L. ETCHELLS and IVAN D. JONES*

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Presented at the Mid-Year Meeting of the National Pickle Packers Association, June 16, 1930, Chicago, Illinois. Paper No. 363 of the Journal Series of the North Carolina Agricultural Experiment Station. The article is based on material summarized chiefly from published papers and bulletins written by the workers connected with the cooperative project on such research. In a few cases, information from abstracts of articles either awaiting publication or prepared for publication has been used.

The formation of relatively small amounts of gas as compared to strong brines. Studies on the composition of the gas from cucumber fermentations have shown that this gas may be carbon dioxide resulting from yeast activity, or a mixture of carbon dioxide and hydrogen resulting from the growth of hydrogen-producing bacteria (3, 4, 5, 6).

Other contributions of the chemical studies are given in the discussion on bloaters.

Bacteriological Studies

The work on the bacteriological changes during the fermentation of cucumbers and other vegetables in brine has done much to reveal the nature of the microbial groups involved and thus gives a better understanding of the fermentation proper. The literature on cucumber brining reveals that although bacteriological studies on this subject started over 50 years ago, the reports until recently dealt principally with the lactic acid fermentation. Our work on cucumbers and other vegetables has shown that what was presumed to be principally a simple lactic acid fermentation is actually a complex group of fermentations. The break-down to date has shown that the following microorganisms may be involved: The lactic acid bacteria; the yeasts; the coliform bacteria; the hydrogen-producing bacteria with an obligate relationship toward salt; and the coccus forms (4, 5, 6, 7, 8, 9, 10, 11, 12).

It has been demonstrated that lactic acid fermentations are restricted chiefly to salt concentrations below 15 per cent (7). Growth by yeasts is found over an extended range both with respect to salt and acid content of brines.
The cooperative pickle research program conducted jointly by the U. S. Department of Agriculture and the North Carolina Agricultural Experiment Station was initiated at Raleigh, North Carolina, in 1935. This program was an expansion of a study which had been started about two years earlier by the Department of Horticulture of the Experiment Station. Besides this cooperative agreement, a similar arrangement was made between the Department of Agriculture and a large pickling plant in eastern North Carolina, so that adequate provisions for work on a commercial scale could be provided.

An indication of the active participation of the different groups cooperating in this project is evidenced by the productivity it has maintained. Since the appearance of the first article in 1938, 35 articles and bulletins have been published on the brining and pickling of cucumbers and other vegetables. Five other articles are either awaiting publication in journals, or are ready to submit for approval for publication. This will make a total of 40 contributions from 1938 through 1950.

The results of the pickle work can be grouped under the following broad classifications of studies: chemical and physical; bacteriological bloater studies; brine-stock firmness studies; pasteurization; sanitation; cucumber varietal studies; softening enzyme studies; and brining and pickling of vegetables other than cucumbers.

The coliforms also have a high salt tolerance but are very sensitive to either added acid or that developed by the lactic acid group (5, 6). The hydrogen-producing bacteria have a high degree of salt tolerance and in addition must have considerable amounts present or they will not grow (8). In this respect they are the only group obtained from brines so far that require salt before they can be cultivated. The coccus forms are very salt-tolerant and predominate in strong brines that are low in acid.

Identification studies have been made on certain of these microbial groups. Because of their direct relationship to bloater formation, rather intensive taxonomic investigations have been conducted on the yeasts. It should first be mentioned that yeasts associated with cucumber brines are divided into two general groups: those which produce a gaseous fermentation in the brine, and those which produce luxuriant films on the surface of brines exposed to air but sheltered from direct sunlight.

Recently, the principal yeasts occurring during the gaseous fermentation of 42 commercial vats of cucumbers were isolated and identified (10). Of the nearly 1,500 cultures identified, almost 90 per cent were placed in two new species, namely, *Tolypopsis caroliniana*, and *Bretanomyces versatilis*. The first yeast predominated during the first 30 days of fermentation, and the second species monopolized the later stage of yeast activity in brines. Species obtained less frequently were *Bretanomyces sphaericus* (new species), *Hansenula subpelliculosa*, *Tolypopsis holmii*, *Torulaspora rosei*, and *Zygosaccharomyces* species. These results were based on fermentations occurring under southern commercial conditions. A study on several hundred yeast cultures from northern brining areas demonstrates that the pattern, with respect to the predominating species, is very similar to that from southern brines but not identical.

Studies have also been made on the types of yeasts responsible for film formation on 40 commercial cucumber brines obtained in five states, (North Carolina, Georgia, Michigan, Indiana, and Wisconsin) (11). The most numerous and widespread film yeast found was the species *Debaryomyces membranefaciens* var. *Hollandicus*. The others found were *Zygosaccharomyces halomembranis* (a new species), *Endomyces obmeri* (a new species), *Endomyces obmeri*, var. *minor* (a new variety), and *Candida krusei*.

Other identification studies on the organisms contributing to fermentation of cucumbers have shown the following: cultures of lactic acid-forming bacteria, typical of a large number of isolates obtained during the acid fermentation under commercial conditions, were found to be *Lactobacillus plantarum* (12). The cultures of coliform bacteria were placed in the species *Aerobacter cloacae*. Studies on the salt-loving, hydrogen-producing bacteria are still in progress; however, a brief description of these

- Dozens of brining vats surround this modern pickle plant.

- Micro-photos of microbial growth types that occur during fermentation of brined cucumbers: (left) rod-shaped cells of the principal species of lactic acid-forming bacteria in brines which is responsible for the acid fermentation; (right) oval cells of one of the brine yeasts responsible for the gaseous fermentation. Both were magnified approximately 1,600 times.

- Yeasts growing below the brine surface often bring about a vigorous gaseous fermentation such as shown above. This type of fermentation generally results in the formation of a high percentage of bloaters (bollow cucumbers).
organisms, obtained from salted corn, has been reported (8).

It has been estimated by leaders in the industry that the formation of bloaters (hollow cucumbers) represents an annual loss of about $1 million. This loss is actually a depreciation in the value of the stock, since cucumbers that have become bloaters cannot be used for the more valuable items like whole pickles, but must be used for relish and cut pickle, which sell for less money.

The research on bloater formation has involved physical, chemical, and bacteriological studies. It has been found that bloater formation is directly associated with the gaseous fermentation occurring during the brine fermentation (3, 10, 13). There are two chief gaseous fermentations responsible for bloater formation; one is caused by yeasts, and the other by salt-loving, hydrogen-producing bacteria. In general, probably the bulk of the bloaters formed result from yeasts. The hydrogen fermentation is sporadic; some years it is completely absent in various brining areas. Furthermore, the hydrogen-producing bacteria are very sensitive to acid formed during the fermentation. They are, therefore, frequently found only during the early stages of the curing period and are stopped by acid formed by the lactic acid bacteria.

A number of factors other than the predominating microbial groups present were found to influence bloater formation in salt stock and dills. These included: the brining schedules; the addition of sugar to the brine; the addition of considerable quantities of organic acids, such as lactic or acetic, during the early stage of curing; and the relative sizes of the cucumbers being brined.

With respect to the relationship between brining treatments and bloater formation, it was found that higher percentages of bloaters were present in lots of salt stock which were produced by the use of high salt-content brines than in lots obtained through the use of initially low salt-content brines (13). This is illustrated by the following results from cucumber curing studies carried on for several seasons under conditions typical of the industry: treatments using brines of 5, 10, and 15 per cent salt by weight resulted in the formation 7, 23, and 44 per cent bloaters respectively. Also, correspondingly greater amounts of gas were obtained with brines of increasingly higher initial salt concentration (2, 6). The composition of the gas from bloated cucumbers was the same as that being evolved from the individual fermentations from which the cucumbers were taken (3, 6).

The influence of the addition of sugar to brines at the start of brining, or during the fermentation of salt stock and genuine dills was investigated. This work was undertaken in view of a number of reports by other workers advocating such practice in order to increase brine acidity. The results of our studies representing a series of experiments conducted over a period of several years definitely proved the following: not only did the use of sugar fail to increase the brine acidity to a useful degree, but it was responsible for a large increase in the percentage of bloaters (90 per cent) as compared to non-sugar added lots (28 per cent) (13, 14, 15). This conclusion held both for the manufacture of salt-stock pickles and genuine dills. Thus, an unnecessary and expensive practice was eliminated.

The addition of considerable quantities of organic acids to brine during the early stages of the curing process favors the formation of increased numbers of bloaters (14, 16). This occurs because the added acid retards the lactic-acid forming bacteria, thereby leaving a larger residue of sugar for utilization by yeasts.

Bloat formation during the sweetening of pickles in the factory (17), and in jars of finished pickles, has also been shown to be the result of a gaseous fermentation by yeasts. In these cases spoilage took place because the amounts of acid or sugar were insufficient to prevent yeast growth. Recently, during an outbreak of spoilage of sweet pickles, a number of very acid-, sugar-, and benzoate-tolerant yeast cultures identified as belonging to the genus *Zygosaccharomyces* were isolated. By testing the ability of these cultures to grow in various concentrations of sugar and vinegar, a very interesting chart has been obtained for predicting the possibility of fermentation occurring during the sweetening operation. When published by T. A. Bell of our laboratory, this chart will be particularly useful for making modifications in commercial formulae with respect to either vinegar or sugar content.
PROGRESS IN PICKLE RESEARCH—II

Pasteurization and softening enzyme studies were among the most significant work carried on during 17 years of pickle research. By JOHN L. ETCHELS and IVAN D. JONES

In the production of salt-stock and genuine dill pickles, firmness of the cucumbers after curing is of prime importance. Lack of firmness is principally the result of a softening action which occurs during curing and/or during brine storage. It is the opinion of many commercial salters and also of several investigators working in the field of brine preservation of cucumbers that the brining schedules followed determine the firmness or lack of firmness of the salt-stock produced.

Numerous commercial and semi-commercial-scale experiments conducted in our studies on the influence of brining treatments on the firmness of cured stocks revealed that there was no treatment tested which would consistently produce salt-stock of a high degree of firmness (18). Likewise, short of gross neglect, no treatment followed would consistently produce soft salt-stock. However, studies now in progress on the influence of certain enzymes on salt-stock firmness are most promising. This subject will be dealt with in greater detail later.

Pasteurization Studies

One of the first publications (1938) from the cooperative pickle project dealt with pasteurization (19). Since then, a number of papers giving consideration to various aspects of the procedure have been released (20, 21, 22, 23, 24, 25). During the past 10 years pasteurization has rapidly assumed a place of major importance in the pickle industry. It is estimated that 1.5 to 2 million bushels of the domestic crop of pickling cucumbers now go into fresh, pasteurized products such as dills and sweet slices. It is the pasteurization procedure that has provided the consumer with a new line of products which were greatly needed to supplement the standard sweets, sours, mixed pickle, and relish. The wholesome appeal of the characteristic crispness and fresh appearance and flavor of the pasteurized, fresh dills and fresh sweet slices makes them welcome and wholly different items in the pickle field. These products have found favor with the consuming public and also with the packer because of the moderate content of sugar, vinegar, and spice employed in their manufacture.

It appears that pasteurized sweet slices were being made by a few companies prior to the start of our work in 1936. However, the details concerning manufacture were presumed to be based on a very secret process which certainly was not available to the industry as a group. The pasteurization treatment that has been developed for the pickle industry was accomplished through the cooperative efforts of the Bureau, the Experiment Station, and one of the large pickle companies in the South. The process has been based on carefully conducted experiments under commercial conditions to determine the exact amount of heat required to kill the types of organisms responsible for spoilage, yet leave the maximum retention of the characteristic crispness and flavor of the fresh cucumber. On the basis of the pasteurization studies, a procedure
adaptable for both continuous and batch operations has been recommended (24, 25). This involves the use of an internal-product temperature of 165 deg. F., followed by prompt cooling, for products containing required amounts of vinegar.

Pasteurization has also been shown to be a desirable practice for improving the keeping quality of genuine dill pickles (26). When properly pasteurized, dills retain most of their original firmness over a storage period of at least a year to 16 months. The same procedure has been recommended for sweet pickle made from salt-stock, which differs from the usual sweet pickle in that it lacks sufficient amounts of sugar and vinegar to prevent fermentation (24). Furthermore, certain large pickle plants are now finding it to their advantage to pasteurize all of their pickle products; both the fresh pack types and those made from brine cured stock (regular sweets, sours, processed dills, and relish).

Information on the pasteurization treatment has reached the pickle industry in a number of ways: through published articles; by talks at national association meetings and at the technical school for pickle and kraut packers usually held annually at Michigan State College; by suppliers’ technical representatives and salesmen calling at pickle plants; by visits of the personnel of the pickle projects to various plants in the country, and by correspondence.

It is our opinion, as well as that of leaders in the industry, that pasteurization of pickle products has been a notable achievement in the pickling field; one which has increased consumption of pickles by reaching new pickle customers. These demands naturally have increased production of the raw product. Also, it has given the packers a standardized procedure for preserving and maintaining the quality of the finished product.

Sanitation

This phase of our work originated from a request of the Sanitation Committee of the National Pickle Packers Association to assist them in developing sanitation rules for cucumber salting stations, and later for factories. This was done after a number of meetings with their Committee, one of which was held jointly with representatives of the Food and Drug Administration in Washington. The rules for salting stations were published on heavy cardboard by the Association and distributed for conspicuous posting at all places where cucumbers were brined. Our suggestions on factory sanitation were presented at the mid-year meeting of the Association at Chicago, Illinois, June 20, 1946, and dealt with premises, manufacturing practices, personnel, and methods of analysis. The talk was later published (27).

Cucumber Varietal Studies

The varietal studies have been an excellent example of cooperation between a research bureau, a major seed company, several state experiment stations, and several processors who are members of the National Pickle Packers Association. All the parties involved in the work were interested in obtaining reliable information on the use of newer and older pickling varieties for salt-stock and for manufactured pickle products. The results so far have shown that definite differences in the brining and pickling qualities exist between varieties. Factors such as shape, firmness, skin-color, and skin-toughness, account for such differences between varieties (28).

Softening Enzyme Studies

Softening of cucumber salt-stock in brine is always a serious economic threat to the industry. The annual loss is estimated at $500,000 to $750,000, but it may be more. Packers do not talk about softening losses as they do about bloaters. Softening losses may be worse in some years than in others, and some brining areas may suffer worse than others. The years 1947 and 1948 were particularly bad in the southeastern states. An estimated 150 vats (700 bushels each) of stock, valued roughly at $250,000, were reportedly either lost or seriously deteriorated in quality during that two-year period.

The availability of brines from soft cucumbers during the summer of 1948 provided one of the most necessary items required to study intensively the nature of this problem. It was found that the softening was due to a pectin-splitting enzyme (chemical agent) which was iden-
tified as similar to polygalacturonate (pectinase). Further, a sensitive method was developed and published for detecting the softening enzyme in brines (29). The examination of several hundred brines, from almost a score of cucumber brining states over the country, has shown that the enzyme is widely distributed from a geographical standpoint.

During the past year alone, 289 vat brines from 21 brining stations, located in nine important cucumber brining states, have been tested for the presence of the salt-stock softening enzyme. In most cases, the salt-stock from these brines was examined for firmness. Of the total number of brines examined for the enzyme, 151 or 52 per cent were positive, while 138 or 48 per cent were negative. There was a very good correlation between positive brines and the soft-texture cucumbers from such brines.

Included in the study for the 1949 season were samples from many brining stations located in northern states. A considerable number of these brines gave a positive softening test. Heretofore it had been the opinion of many that salt-stock softening was a difficulty encountered only in the more southern areas.

Furthermore, for the first time, the softening enzyme has been found by our co-worker, T. A. Bell, in genuine dill pickle brines, and its presence was directly related to the loss of firmness of the dills. The softening occurred in three widely separated states (California, Wisconsin, and New Jersey).

More recently we have been concerned with the origin of the softening enzyme (polygalacturonase) in brines. It is recognized that there are two possible sources: microbial activity during the brine fermentation, and the green cucumber itself. Studies on the latter possibility have shown the enzyme to be present in dry cucumber seeds, staminate (male) and pollinated pistillate (female) cucumber flowers, and ripe cucumbers. The enzyme was not found in the unpollinated pistillate flowers, leaves, petals, and stems (30). Studies were also made on the tomato for comparative purposes. The results showed that the red-ripe tomatoes contained the softening enzyme but it was not found in the embryos with flowers or in the green tomatoes.

Study Quick-acting Enzyme

Another pectin-splitting enzyme has been studied intensively as to its possible source of origin in cucumber brines (31). It is called pectinesterase (pectase), and its potential importance stems from reports by workers in the Bureau's enzyme division at Albany, California, and others, that it speeds up the action of the softening enzyme discussed earlier. The chief source of the de-esterifying enzyme, pectinesterase, in the brine appears to be the cucumber (31). It has been found in the dry seeds, the leaves, petals, stems, flowers, and the cucumber fruit. With the cucumber during development this enzyme remains at a fairly constant level. This is in contrast to the tomato where the enzyme content increases rapidly to over 300 times the initial amount. In commercial cucumber brines, the enzyme (pectinesterase) decreases during the fermentation and storage period. The loss has been attributed chiefly to the inhibiting action of the brine acids on the enzyme.
The possibility of microbial activity during fermentation being a source of the softening enzyme (polygalacturonase-like) has also been investigated (32). Because of their general prevalence and of their high tolerance to salt and acid, brine yeasts seemed to be a reasonable starting point. A total of 143 yeasts, representing 66 species in 15 genera were screened as to their ability to hydrolyze citrus pectin. This total included representative species of the yeasts responsible for the gaseous fermentation of brined cucumbers, as well as those responsible for film formation on brines. Thirty-three of the species tested came from other collections.

None of the yeasts from cucumber brine origin was found to be a potential source of the softening enzyme. However, most of the film-forming species were capable of de-esterifying pectin, but did not hydrolyze it further. Four yeasts from sources other than cucumber brines were capable of producing the softening enzyme. One of these came from spoiled citrus concentrate (33). This may be of value to those concerned with the manufacture and keeping quality of those citrus products which are not heat-processed.

The enzyme studies have been aided by intra-bureau cooperation with the Enzyme Division. E. F. Jansen of that division visited our laboratory for a week during January, 1950 and gave the writers and Mr. Bell the benefit of his years of experience in the field of pectic enzymes. At present our laboratories are working cooperatively with the Enzyme Division on certain fundamental aspects of the work.

Wartime Studies

From early 1942 until 1945 our research efforts turned to the emergency preservation of food and feed. The cucumber brining and pickling work was replaced by similar studies on other vegetables. During 1942, 87 vegetable fermentations were followed with respect to bacteriological changes, supplemented with chemical, physical, nutritive value, and keeping quality observations. The vegetables investigated included green beans, green peas, green lima beans, wax beans, white corn, yellow corn, butter beans, lettuce, carrots, tomatoes, celery, okra, and certain leafy vegetables.

As fast as possible, the results on salting and brining of vegetables were translated for practical use in the home and in industry. Farmers’ Bulletin No. 1932, released in 1943, contained recommendations for salting and brining a number of vegetables on both home and commercial scale (34). There was a heavy demand for this bulletin, and requests for over 350,000 copies were received during the first six weeks after it was released. The bulletin was revised in 1944 and the commercial application deleted.

Several other articles dealing with the preservation of vegetables by salting and brining were published. These included: technical bulletin on bacteriological changes (8); journal articles on nutritive value studies (35, 36); and, general articles on the preservation method (37, 38). In addition, prior to the publication of the farmers’ bulletins, three mimeographed publications were released (AIC-4, May, 1943; AIC-4, rev. July 1943; AIC-4, rev. August, 1943).

Information gathered during the 1943 season revealed that several million pounds of vegetables, such as corn, green peas, green beans, celery, and okra were salt-preserved by commercial concerns for use in food products. Substantial amounts were also preserved in the home by this method.

Summary and Conclusions

Some of the contributions to the knowledge of commercial brining of cucumbers, resulting from the cooperative investigations of the Bureau of Agricultural and Industrial Chemistry of the U.S. Department of Agriculture and the Department of Horticulture of the North Carolina Agricultural Experiment Station, have been presented.

Studies reported during the period from September, 1938 through June, 1950 have, in part, accomplished or demonstrated the following:

1. The basic chemical changes taking place in the brine during fermentation have been charted with respect to development and types of acids, pH, sugar utilizations, development of alcohols and other solvents, and the amount and kinds of gases evolved.

2. The initial use of low salt brines gave rapid formation of a high amount of acid and a low pH. Increasingly higher initial brine strengths gave slower rates of acid formation, and lower amounts of acid.

3. Fermentations at low brine strengths gave relatively small amounts of both gas and bloaters as compared to fermentations in high salt brines.

4. The composition of the gas from cucumber fermentations has been shown to be carbon dioxide from yeast activity, or a mixture of carbon dioxide and hydrogen resulting from the growth of hydrogen-producing bacteria.

5. Numerous experiments conducted on commercial and semi-commercial scale have definitely proved that there is no one brining treatment which consistently gives the same quality of firmness of salt stock from one year to another.

6. The work on the bacteriological changes during fermentation has done much to reveal the nature of the microbial groups involved and thus give a better understanding of the fermentation proper.

7. The principal groups of micro-organisms that may be involved in the fermentation are the lactic acid bacteria, the yeasts, the coliforms, the salt-loving, hydrogen-producing bacteria, and the coccus forms.

8. Identification studies have been done on the yeasts responsible for the gaseous fermentation, the yeasts responsible for film formation on brines, the lactic acid bacteria, and the coliforms.

9. Bloater formation in both salt-stock and finished pickles has been shown to be the result of gaseous fermentation, and the various factors influencing this type of fermentation are discussed. Yeasts are considered to be responsible for most bloaters.

10. Pasteurization has been a notable achievement in the pickling field; one which has increased consumption of pickles by reaching new customers with new and inviting products. Also, pasteurization has given packers
a standardized procedure for preserving and maintaining the quality of the finished product.

11. Assistance has been given the Research Committee of the National Pickle Packers Association in developing sanitation rules for operation of salting stations, and factories.

12. Cucumber varietal studies have shown that definite differences in the brining and pickling qualities exist between varieties.

13. The softening of cucumber salt-stock and genuine dills under commercial conditions has been shown to result from action by a pectin-splitting enzyme identified as being similar to polygalacturonase. A sensitive test has been developed for detecting the softening enzyme in brine.

14. Two principal possibilities as to the source of the softening enzyme in brines exist: The microbiological activity occurring during curing and storage of cucumbers in brine; and the green cucumber that is brined. First results on studies in both of the above categories are reported.

Acknowledgments

A number of people have contributed notably to the progress of the projects described above. Among these are Dr. M. K. Veldhuis, Dr. Otto Veerhoff, and Martin A. Hoffman, who formerly were closely associated with the project and were responsible for certain phases and progress of the work.

Thomas A. Bell is responsible for the enzyme studies. He is assisted by Mrs. Mary Guy and Miss Eleanor Gibbs, William M. Lewis, Charles S. Sullivan, Robert R. Robinson, Jr., and Robert Tilley, while students at North Carolina State College, aided materially in the field and laboratory operations.

The late Harvey B. Ohmer, formerly of the Chemistry Department, did much to further the work, both while working during the summers from 1937 to 1941 as bacteriologist at the Charles F. Cates Company, and while taking graduate work in brine fermentations at North Carolina State College.

Appreciation is expressed to M. E. Gardner, Head, Department of Horticulture, North Carolina State College, for cooperation and assistance which has been invaluable in these studies; to F. B. Faust, Associated Seed Growers, Inc., Atlanta, Georgia, and to the many pickle companies located in both northern and southern brining areas for facilities and materials generously supplied.

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33. H. H. Hall. (Personal communication.)


* A complete list of publications is available from Food Fermentations Laboratory, Box 5578, Raleigh, N. C., or to the Department of Horticulture, North Carolina State College, Raleigh, N. C. Ask for AIC-183.