V.2 Factors Influencing the Growth of Lactic Acid Bacteria During the Fermentation of Brined Cucumbers

J. L. ETCHELLS, H. P. FLEMING AND T. A. BELL

Food Fermentation Laboratory,
Southern Region, Agricultural Research Service,
United States Department of Agriculture, and Department of Food Science,
North Carolina State University,
Raleigh, North Carolina 27607, USA

Introduction

In the United States, the manufacture of cucumber pickles is a sizeable economic enterprise (Table I). Of the average annual harvest, approximately 40% is made directly into fresh-pack or pasteurized pickle products such as whole dills, dill spears, sweet slices, dill chips, etc. This process is essentially a canning operation which has grown to its present proportions in this country since our early studies which defined the principles of the process (Etchells, 1938; Jones *et al.*, 1941; Etchells and Jones, 1942). Prior to this time, practically all of the cucumber crop was brine-cured; including genuine dills in barrels. Now, the remaining portion of the crop, 60%, is converted into brine-stock pickles by natural fermentation in brine. The cured brine-stock is desalted and manufactured into various staple pickle products such as sweets, sours, mixed pickles, processed dills, hamburger slices, relishes, etc. Substantial amounts of other commodities are brine-cured in this country each year, including Spanish-type green olives (4500 tons), citron (5000 tons), onions (3000 tons), and lesser amounts of peppers, cauliflower, green tomatoes, and okra.

TABLE I

Information on production and value of pickling cucumbers in the United States — 1972 season [1]

Item [2]	Amount and value of crop
Harvested acreage	128,830 acres (about 52,760 hectares)
Yield per acre	4.43 tons; 177 bushels (4.02 metric tons; 4,021 kg)
Amount of crop harvested	571,000 tons; 23 million bushels (511,000 metric tons)
Price per ton and per bushel	$94/ton; $2.35/bu (field run)
Value of crop to farmer	About $60 million (dollars)
Value of crop at manufacturer level	About $400 million (dollars)
Value of crop at retail level	About $500 million (dollars)
Per capita consumption of pickles	About 7.9 lb. (3.6 kg)

[1] Information supplied by Pickle Packers International, Inc., St. Charles, Illinois 60174.

[2] Tonnages shown represent the 'short ton' = 2000 lb = 0.90718 metric tons. Also, 1 bushel of cucumbers was figured at 50 lb or 22.7 kg; and, a hectare was calculated at 2.47 acres.

Objective

The primary goal of our laboratory since its origin, over 30 years ago, has been to develop a feasible, controlled bulk fermentation process that would reduce or eliminate the major defects and spoilage problems encountered in brining. Although we have made a number of contributions which industry has adopted over the years (Etchells and Moore, 1971), only recently, have we developed a process which we believe offers a practical means for complete quality control of the fermentation of cucumbers brined in bulk containers. The process combines a prompt, vigorous lactic acid fermentation, favoured by the introduction of starter cultures, with the control of undesirable microbial activity and fermentation gases.

The controlled fermentation process was the culmination of intensive studies of the heterogeneous, highly variable, and competitive microbial groups that are present in the natural fermentation of cucumbers as practiced during commercial brining. This paper summarizes part of the findings from many years of cooperative research of our laboratory with some 50 co-authors, not only from the pickle industry, but also from various state and federal research agencies. Finally, we have summarized the controlled fermentation process in what might be called a 'flow sheet'. We have found that the procedure outlined usually results in a predictable fermentation that produces consistently superior quality brine-stock pickles. The two references cited with the 'flow sheet' should be consulted for the full details of the procedure to carry out the controlled fermentation process step by step.

Factors Affecting the Lactic Acid Fermentation of Cucumbers

In the preservation of cucumbers by brining, the fermentable sugars preferably should be utilized primarily by homofermentative lactic acid bacteria with the exclusion of micro-organisms which cause quality defects in the product. In natural fermentations as practiced commercially, however, the heterogeneous microbiological activity leads to wide variation between fermentations and to various types of deterioration and spoilage of the brine-cured stock such as:

Bloater formation (internal cavities due to excessive gas production)
Flat, shrivelled and distorted stock (due to gas pressure)
Loss of texture or firmness
External and internal bleaching; other 'off' colours
Unclean or offensive odour and taste.

There are many highly complex, variable and interrelated factors which influence the direction of the fermen-

tation. Brining treatments, environmental conditions and initial microbial populations are primary factors which decide the course of microbial activity. Properties of green stock are directly related to the quality of the brine-cured stock; but, in addition, characteristics of the fruit itself have some effect on the nature of the fermentation.

Brining and Environmental Conditions

Green cucumbers are usually brined in wooden tanks ranging in capacity from 100 to 1000 bushels. After the tanks are properly filled, they are fitted with a loosely constructed, 'false' head or cover, made of wooden boards about 1 in. (2.54 cm) thick, and keyed down securely by heavier wooden timbers. Salt brine of a suitable concentration is then added to a level of a few inches above the head. Next, dry salt is added on the cover to maintain the initial brine concentration, which otherwise would be diluted by the water content of the cucumbers (approx. 95%). Results of a typical natural fermentation (under northern USA brining conditions) are represented in Fig. 1.

The initial brine strength may vary, depending on the individual pickling company. In earlier days, higher brine strengths were used, particularly in southern areas, because high salt levels were believed to retard softening of the cucumbers, especially the smaller sizes (up to 1¼ in. diam.). It was later found that the softening action was due to pectinolytic enzymes contained in highly mould-laden cucumber blossoms that were retained on the small fruit (Bell, 1951; Etchells *et al.*, 1958). Retention of blossoms was greatest on small fruit. Further, blossom retention was more common early in the harvest season (June, in North Carolina) as compared to the latter part of the intake (July). Also a study in several other states during harvest showed cucumber varieties differed as to flower retention (Bell *et al.*, 1958). By draining the tanks of the initial cover brine after 36-48 h, sufficient amounts of the diffused enzyme were removed to eliminate the softening problem (Etchells *et al.*, 1958). After industry adopted the draining

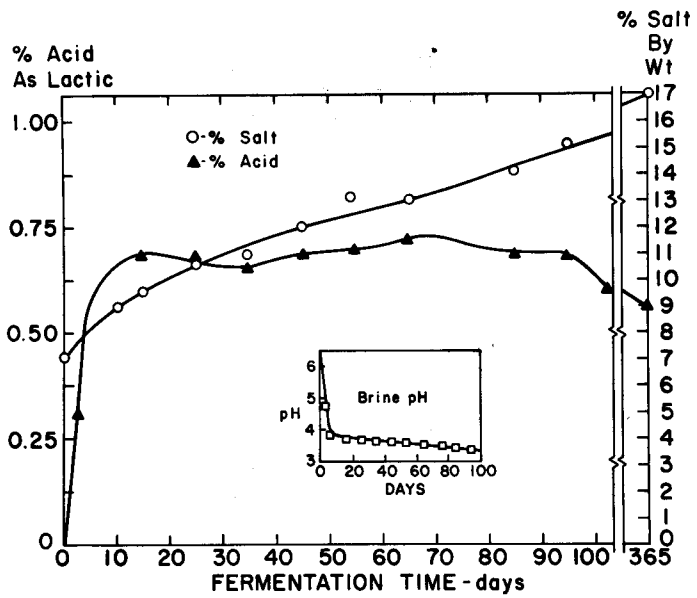

Fig. 1. The natural fermentation of brined cucumbers. Values represent averages of brine samples from 155 fermentations. From Etchells *et al.* (1952). (Brined under northern USA conditions.)

procedure and, as their fear of softening abated, the trend in recent years has been toward lower brine concentrations—those in the 20-30° salometer (sal.) range (5-8% NaCl) *after equilibration*. (The pickle industry expresses salt concentration in degrees salometer which is % saturation with respect to NaCl by wt; 100° sal. = 26.4% NaCl at 60° F. For convenience of the reader, the relationship between salometer and % salt by weight is shown in Fig. 2.) Reduction of the brine strength was also very helpful in reducing the production of bloaters (hollow stock) in the larger sizes, 1½-2¼ in. dia. Draining has been a satisfactory method of dealing with the softening problem since 1954; but recently, state and federal regulations concerning disposal of salt have prompted our search for other solutions. Extensive studies have shown that use of an inhibitor of pectinolytic and cellulolytic enzymes, which has been isolated from various plant sources, can control softening of brined cucumbers (Bell and Etchells, 1958; Bell *et al.*, 1962; 1965b). The forage crop, *Sericea Lespedeza*, is a particularly rich source of the inhibitor; 50-100 ppm of a crude extract therefrom will block enzymatic softening of brined, no. 1 size cucumbers (Bell *et al.*, 1965a). However, there has been difficulty in obtaining approval for use of the enzyme inhibitor in commercial brining tanks. More recently, we have been able to remove the softening enzymes from the brine of no. 1 size stock and reclaim

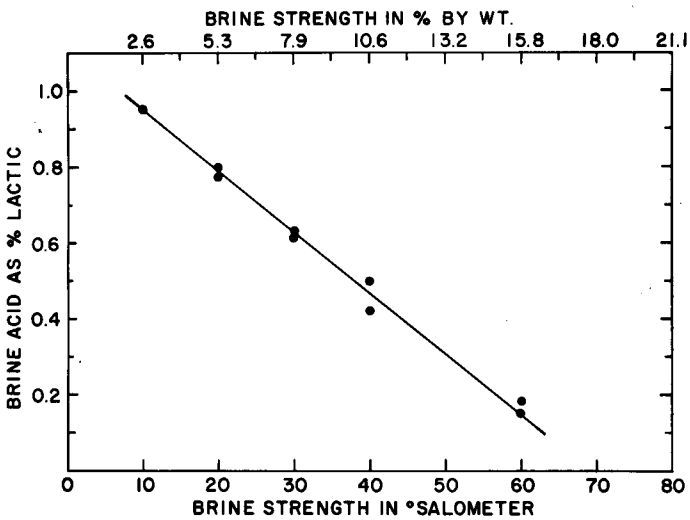

Fig. 2. Influence of brine strength on developed acidity in naturally fermented cucumbers. Values represent averages of 28, 85-bushel lots over a four-year period (Jones and Etchells, 1943) and numerous commercial tanks located in southeastern USA.

the salt brine for further use. Results of this study will be made available to industry in the very near future.

With equilibrated brine strengths in the 20-30° salometer (5-8% salt) range, and with brine temperatures in the 24-30° C (75-85°F) range, salt-tolerant micro-organisms grow actively for about 4-6 weeks. Certain species of yeasts discovered by the authors may remain viable in stored brine-stock for a year or more (Etchells *et al.*, 1952). Lactic acid is produced during the active fermentation and the brine strength is gradually increased by the addition of dry salt on the head of the tanks until a final holding strength of 60-70° salometer is reached. The salt strength of the brine during the early part of the brining process must be carefully controlled to permit a desirable level of acid production. Higher brine strengths result in lower acid production (Fig. 2). Therefore, it has been recommended (Etchells and Hontz, 1972) that a minimum of 0.6% acid be present prior to raising the brine strength. A quick, simple test was developed to help the pickle industry determine when this level of acidity has been reached (Bell *et al.*, 1971). This test is called 'Q-BAT ™', meaning 'Quick Brine Acidity Test'. The brine strength then may be raised according to a schedule. An increase of 5° salometer during a week is suitable in southeastern and southern parts of the United States, where brine temperatures from mid-May to late September and often to mid-October are generally favourable for good growth of lactic acid bacteria.

This schedule usually permits production and brining of two crops of pickling cucumbers in North Carolina growing areas and in other production areas in comparable temperature zones. In cooler climates, found in northern areas of the USA, especially in the Great Lakes Region, the brine strength may not be raised according to any set schedule but is dependent mostly on early-season temperatures to enhance the progress of the acid fermentation. Here, sugars in the stock should be fermented as completely as possible before cold weather approaches and the brine strength is increased to preclude freezing; otherwise, in the spring, fermentative yeast species may use the residual sugars to produce a vigorous gaseous fermentation; usually accompanied by serious bloater damage (hollow stock). Thus the procedure of controlled fermentation of brined cucumbers, to be discussed later, should be of particular interest to briners in northern areas.

TABLE II

Microbial counts on pickling cucumber fruit—Model variety no. 1 size for three seasons

Microbial group	Colony counts [1,2] 1st season	2nd season	3rd season
	thousands/g	thousands/g	thousands/g
Bacteria			
Aerobes			
Total	16,000.0	3580.0	3260.0
Spores	17.0	3.0	3.3
Anaerobes			
Total	1830.0	187.0	246.0
Spores	0.8	0.13	0.13
Coliforms	3940.0	730.0	660.0
Acid-formers	5.0	5.3	13.2
Yeasts	1.6	1.0	0.7
Moulds	3.4	3.1	1.6

[1] Colony counts shown represent the average of seven weekly samplings for the first season; and four each for the second and third seasons. The average single fruit weight (unit) for the first season was 12.4 g (= ¾ - ⅞ in. dia.); for the second and third, about 20 g(= ⅞ - 1 in. dia.). Thus, to obtain estimates of the microbial populations on a per fruit (= unit) basis, the populations should be multiplied by 12 for the first season and by 20 for the second and third seasons.

[2] Data shown for the first season are based on results of Etchells *et al.* (1961b; for the second and third seasons, from Etchells *et al.* (1973a).

TABLE III

Microbial populations on cucumber fruit and blossoms

Microbial group	Colony counts [1,2] Cucumber fruit Per g	Per unit [2]	Cucumber blossoms Per g	Per unit
	thousands	thousands	thousands	thousands
Bacteria				
Aerobes				
Total	16,000.0	182,320.0	18,200,000	476,000
Spores	17.0	218.0	67,800	1,940
Anaerobes				
Total	1830.0	19,800.0	3,092,000	78,760
Spores	0.8	9.8	2,100	191
Coliforms	3940.0	49,125.0	6,400,000	167,530
Acid-formers	5.0	60.0	26,000	765
Yeasts	1.6	18.0	3,030	82
Moulds	3.4	44.0	11,300	295

[1] Counts shown represent the average for 7 samplings of no. 1 size Model variety cucumbers and 2 samplings of blossoms collected during the 1st season.

[2] Average unit weights: cucumber, 12.4 g; cucumber blossom, 0.0275 g. Data shown are based on results of Etchells *et al.* (1961b).

Microbial Populations and Interactions

Microbes that cause the fermentation come chiefly from the cucumbers and adhering particles of soil. They consist of various groups of bacteria, yeasts and moulds (Tables II and III). Numbers and relative proportions of these groups vary considerably between seasons and within the *same* season. Of the total population of microorganisms present, lactic acid bacteria constitute an ex-

TABLE IV

Influence of the week of harvest on populations of acid-forming bacteria on Model variety, no. 1 size, pickling cucumber fruit

Week of Harvest	Acid-forming bacteria: colony count[1] per Gram	Fruit
	thousands	thousands
1	0.8	16
2	1.6	32
3	4.7	94
4	15.7	374

[1] Values shown based on two harvest seasons for small size fruit with average weight of about 20 g. Data shown are based on results of Etchells *et al.* (1973a).

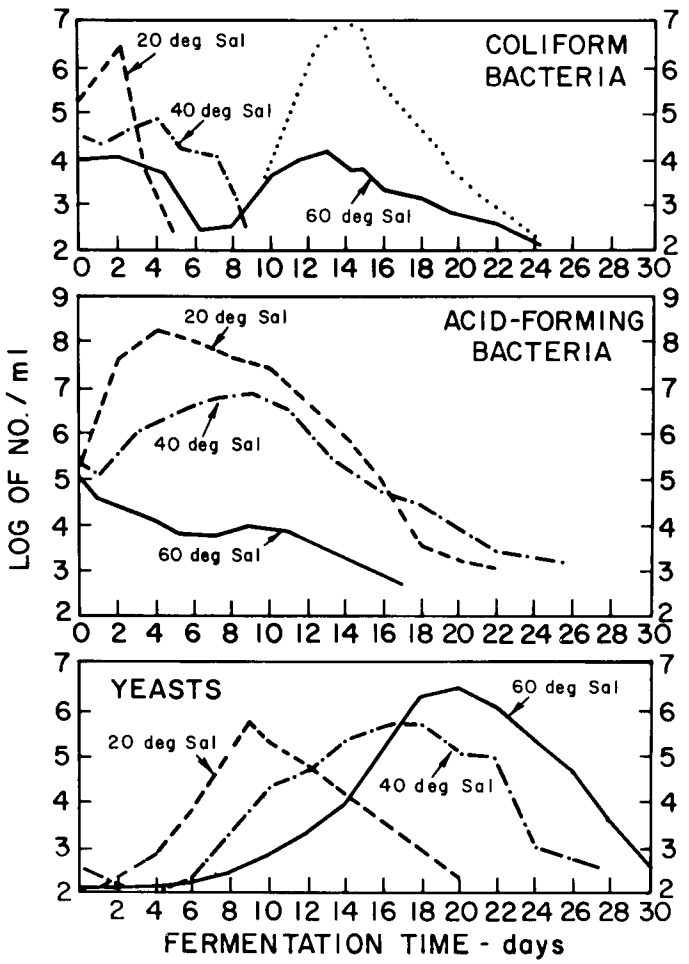

Fig. 3. Effects of brine strength on the predominating microbial groups in natural fermentations of brined cucumbers. From Etchells and Jones (1943).

tremely small number, and this number is subject to seasonal variations (Table IV). The initial microbial load that enters the brining tank on the cucumbers may vary due to prebrining treatments. The fruit may be brined the same day of harvest, or may be brined after delay of a day or so either in transit from distant production areas or at the brining station. Cucumbers are usually *not* washed before brining, except for special reasons such as being extremely dirty or for controlling the spread of plant parasites such as witchweed seed. Cucumbers for genuine dills or overnight dills should receive a thorough washing as they are usually eaten from the original container or repacked. This applies to all container sizes, ranging from barrels to quart glass and the like.

The type of fermentation, as to ascendancy and predominance of a given microbial species and resulting end-products, is influenced to a great extent by initial brine strength and the rate at which it is increased. Aerobic and anaerobic spore-forming bacteria are usually inhibited, by the concentration of salt and the pH of the cover brine, and never become established. Growth curves of the three major salt-tolerant microbial groups active in three experimental brine fermentations are shown in Fig. 3.

Coliform bacteria, which occur in the highest numbers among the three groups on the green fruit (Table II), become established promptly in a low-strength brine such as 20° salometer. Although one type of coliform bacteria is inhibited at higher brine strengths, 40-60° salometer (10.5-16% NaCl), another, a halophilic group (see dotted line, Fig. 3), similar to species in the genus *Aerobacter*, may become actively established at these concentrations, especially if lactic acid bacteria have not produced brine acid at inhibitory levels. Activities of both types of coliforms are characterized by high evolution of carbon dioxide and hydrogen. The coliform bacteria and related Gram-negative species are readily inhibited by the brine acid resulting from prompt growth of the lactic acid bacteria at about 40° salometer and below. At 60° salometer, the halophiles and the yeasts use the brine nutrients and usually produce copious quantities of gas (carbon dioxide and hydrogen) and a high percentage of bloated stock (hollow).

Two groups of yeasts active in natural cucumber fermentations are the subsurface (fermentive) types which usually cause a gaseous fermentation, and the surface or film (oxidative) types.

Although yeasts are present in very low numbers on the raw cucumber fruit (Table II), the high tolerance of subsurface yeasts to salt and brine acid permits them to grow in the brine even after the lactic acid bacteria have been inhibited by low pH (pH 3.1-3.2), *providing brine sugars are available.* In one instance, yeasts (*Brettanomyces*) were active in the brine for months (Etchells *et al.*, 1952). In high salt treatments, the problem is even more acute as the lactic acid bacteria are unable to convert much sugar to acid (Fig. 4) and leave more sugar for the yeasts (Fig. 3). Evolution of large quantities of gas (carbon dioxide) is evidence of the *fermentative type* of yeast, as is shown in the 60° salometer fermentation (Fig. 4). The principal species of subsurface yeasts, based on our identification of several thousand isolates, listed in the approximate order of their frequency of occurence in cucumber brines are: *Brettanomyces versatilis, Hansenula subpelliculosa, Torulopsis caroliniana, Torulopsis holmii, Saccharomyces rosei, Saccharomyces halomembranis, Saccharomyces elegans, Saccharomyces delbrueckii, Brettanomyces sphaericus,* and *Hansenula anomala* (Etchells *et al.*, 1961a).

Surface yeasts create an entirely different problem. These species grow luxuriantly on the surface of brines of sheltered tanks, but are controlled effectively by exposure to direct sunlight. Growth of film yeasts was unrestricted for the natural fermentations incubated at three temperatures (Fig. 5). These yeasts oxidized lactic acid resulting in a pH rise to the point where coliform and lactic acid bacteria renewed their growth. In addition to lowering brine acidity, a heavy growth of surface yeasts may

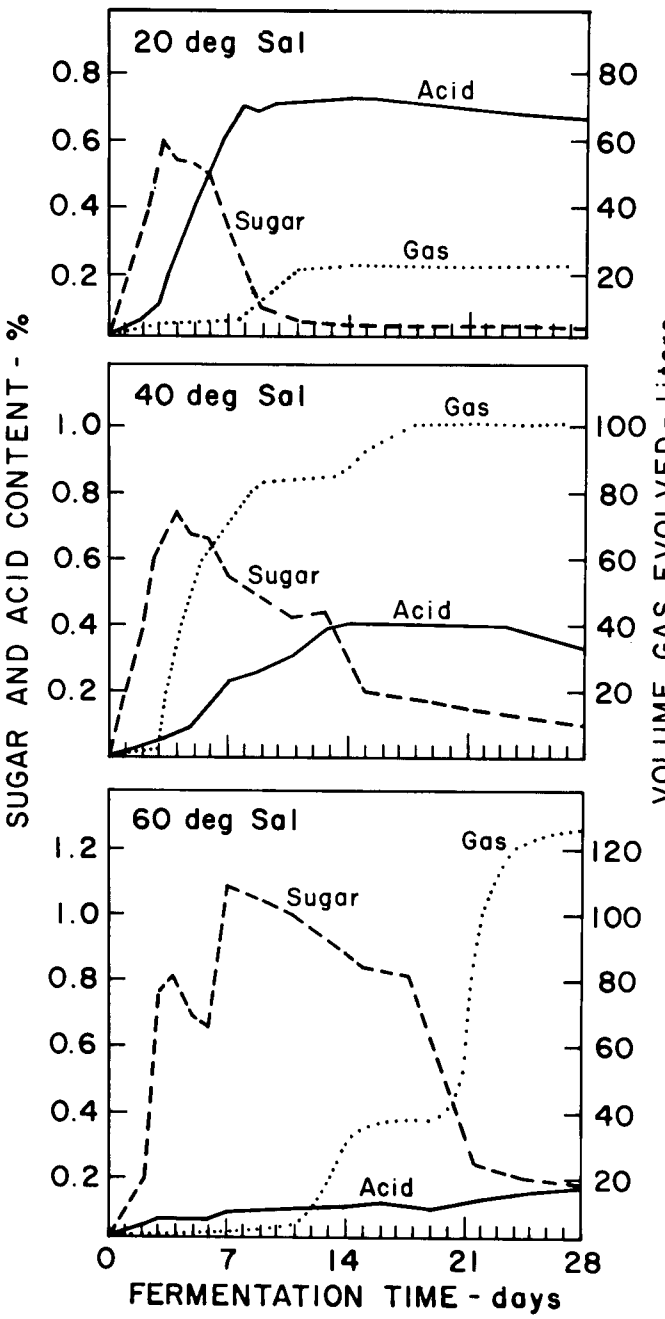

Fig. 4. Effects of brine strength on chemical changes in natural fermentations of brined cucumbers. From Jones and Etchells (1943).

support growth of moulds, putrefactive bacteria, and insect larvae, all of which, if allowed to develop unrestricted, cause a bad odour, a most unsanitary condition, and probably spoilage. The principal species of surface yeasts isolated from commercial cucumber brines include: *Debaryomyces membranaefaciens* var. *Holl., Endomycopsis ohmeri, Zygosaccharomyces halomembranis,* and *Candida krusei* (Etchells and Bell, 1950). Species in the genus *Debaryomyces* were considered by Mrak and Bonar (1939) responsible for film formation on seven samples of brine-stock pickles they examined.

Although lactic acid bacteria are present in very low numbers on green cucumbers (Tables II and III), they quickly become established in brines of approx. 20° salometer (Figs 3 and 5). With the coliform bacteria inhibited early in the fermentation by acid, yeasts remain the major microbial group with which the lactic acid bacteria

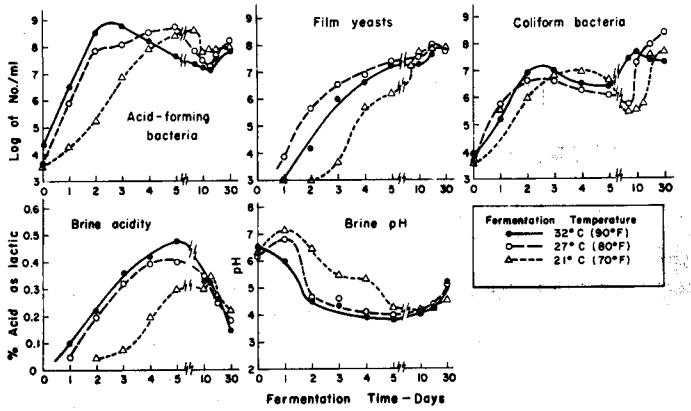

Fig. 5. Microbiological and chemical changes in the natural fermentation of brined cucumbers under sheltered conditions, fermented at three temperatures. Equilibrated brine strength during fermentation, 5.5-5.9% NaCl. From Etchells, Costilow, Anderson and Bell (unpublished).

must compete for fermentable nutrients. Species of lactic acid bacteria that have been isolated from natural fermentations in small containers under laboratory conditions include, in order of increasing prevalence: *Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae, Lactobacillus brevis,* and *L. plantarum* (Pederson and Albury, 1950). Under conditions typical of the pickle industry, however, we have found that the latter three species are the ones usually involved in the commercial fermentation; particularly *L. plantarum* and *P.. cerevisiae.*

Salt-tolerant pediococci grow early in the fermentation, but, depending on the salt content, acidity and brine temperature, are then succeeded by the more acid-tolerant *L. plantarum* and *L. brevis.* The latter two species may grow throughout the fermentation (Costilow *et al.,* 1956), although *L. plantarum* is by far the predominant species in most fermentations (Etchells and Jones, 1946; Rosen and Fabian, 1953). *L. plantarum* dominated the final stages of fermentations, even when cultures of other species were added (Pederson and Albury, 1961). This fact probably is due to the greater acid tolerance of *L. plantarum,* which varies even among strains of this species (Fleming and Etchells, 1968). Only the homofermentative species, *P. cerevisiae* and *L. plantarum,* are desired in the fermentation as high carbon dioxide production by the heterofermentative *L. brevis,* particularly at low brine strengths, can cause serious bloater damage (Etchells *et al.,* 1968b). Variations in salt concentrations and brine temperatures influence the prevalence of the species of lactic acid bacteria (Pederson and Ward, 1949; Pederson and Albury, 1950). For example, low temperatures (45-50°F; Pederson and Albury, 1950) and low salt concentrations (2.5-3.7%; Pederson and Albury, 1956) tend to foster growth of *Leu. mesenteroides.* This species would not normally be encountered in commercially brined cucumber fermentations (6-8% salt) at 24-30° C (75-85° F) for salt-stock purposes.

Properties of the above lactic species have been studied in pure-culture fermentations. Gamma irradiation, as well as heating in hot water (heat-shock), have been used to rid the raw fruit of the naturally occurring asporogenous microflora (Etchells *et al.,* 1961b, 1964). *L. plantarum, P. cerevisiae* and *L. brevis* all grew well in pure culture, attaining maximum populations after about two days at 32° C (Fig. 6). *L. plantarum* produced the most acid and resulted in a minimum pH of approx. 3.3, while *P. cerevisiae* stopped growth at approx. pH 3.7 in brined cucumbers. These two species produce mainly lactic acid with only small amounts of carbon dioxide, acetic acid and other end-products. *L. brevis* produces lactic as well as acetic acid, a relatively high amount of carbon dioxide, ethyl alcohol, and traces of other compounds.

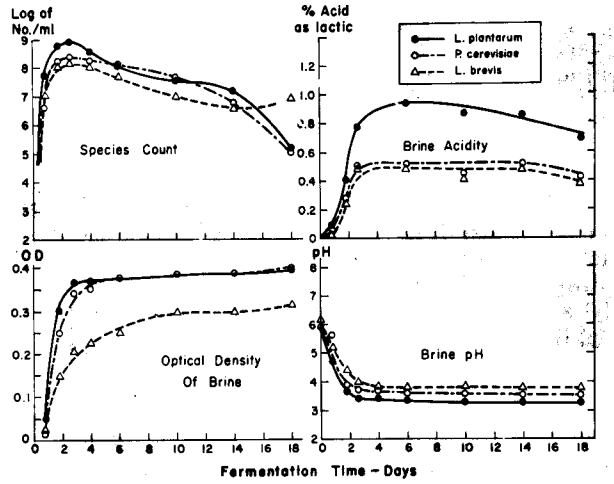

Fig. 6. Pure culture fermentations by three species of lactic acid bacteria in cucumbers brined at 5.3% NaCl. Incubation was at 32° C. From Etchells, Costilow, Anderson and Bell (unpublished).

The above species of lactic acid bacteria, with the exception of *Leu. mesenteroides,* grew in salt concentrations up to 8%, although acid production and microbial counts were lower at the higher concentrations (Table V). Lower acid production at higher brine strengths was particularly noticeable for *P. cerevisiae* as compared to *L. plantarum*

TABLE V

Influence of salt on the growth of four species of lactic acid bacteria in pasteurized cucumbers[1]

Species	Salt concentration (% by wt)	Maximal Plate Count Millions /ml[2]	Age in days when reached	Final brine acidity at 25 days (% lactic)
Pediococcus cerevisiae, FBB-61	0.	1420	2	0.44
	4.2	1430	2	0.21
	6.3	930	2	0.16
	8.1	620	4	0.04
	10.2	< 1[4]	25	0.00
Lactobacillus plantarum, FBB-67	0	2200	4	0.84
	4.2	910	4	0.72
	7.2	600	4	0.56
	8.3	80	4	0.30
	10.3	6	20	0.05
Lactobacillus brevis, FBB-70 and L-544[3]	0	905	2	0.24
	2.1	850	2	...
	4.2	470	2	0.22
	6.2	293	6	0.27
	8.3	29	6	0.17
	10.3	< 1[4]	25	0.01
Leuconostoc mesenteroides, FBB-42[5]	0	1070	1	0.44
	4.2	113	2	0.23
	6.3	6	2	0.13
	8.1	< 1[4]	14	0.06
	10.2	< 1[4]	14	0.05

[1] Tabulated from data of Etchells *et al.* (1964) and Etchells *et al.* (1968a).

[2] Initial counts after inoculation were approx. 1-million cells/ml.

[3] Counts are the average for two strains.

[4] Populations did not increase beyond the initial count during the time indicated in days (4th column).

[5] Tests for this species run separately.

and *L. brevis*. This fact probably accounts for the lower acid production resulting when natural fermentations occur at higher brine strengths (Fig. 2).

Mixed-culture innoculations of cucumbers using a combination of three species showed that growth of *L. plantarum* was delayed (Fig. 7). Further studies revealed that *P. cerevisiae* was responsible for delayed growth of *L. plantarum*, and actually resulted in a marked reduction in cell counts of that species during early stages of fermentation. In other fermentations, *P. cerevisiae* caused about

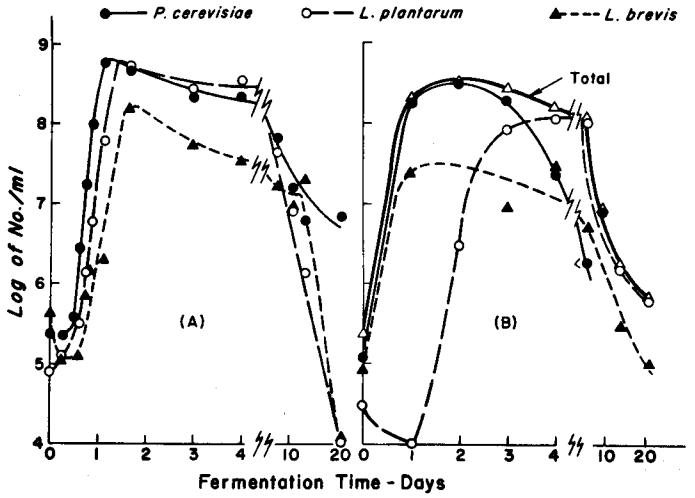

Fig. 7. Growth of *Pediococcus cerevisiae*, *Lactobacillus plantarum*, and *L. brevis* during pure culture fermentation of brined cucumbers. Part A reflects their growth in individual fermentations, and part B, their growth in a single fermentation inoculated with all three species. From Etchells *et al.* (1964).

a week or more delay in growth of *L. plantarum* as indicated by acid production (Table VI). It is conceivable that the inhibitory effect of *P. cerevisiae* may be the basis of the sequence of lactic species noted in natural fermentations. Recent studies have shown that only certain strains of *P. cerevisiae* are inhibitory to *L. plantarum*. This phenomenon is under investigation.

Properties of the Cucumbers Brined

Juice extracted from immature cucumbers has been used to grow various species of lactic acid bacteria (Fleming and Etchells, 1967). It provides an excellent growth medium with no apparent nutritive deficiences for the lactics we have dealt with over the years. Analyses of cucumber juice have revealed an adequate supply of the essential vitamins—biotin, niacin and pantothenic acid—for the growth of *L. plantarum* (Rosen and Fabian, 1953). The amino acids—leucine, isoleucine, valine, tryptophane, glutamic acid and cysteine—were also found in adequate supply (Costilow and Fabian, 1953).

Pickling cucumber varieties contain approx. 2% (±0.2) reducing sugars, and larger sizes contain more than smaller (Jones and Etchells, 1943). These sugars are primarily glucose and fructose (Fleming *et al.* 1973b). It is desirable that all of the sugars be fermented by the homofermentative lactic acid bacteria, as any residual amounts in the brine after growth of *L. plantarum* has been inhibited by its low pH (3.1-3.2) will be utilized by species of acid- and salt-tolerant yeasts. Gaseous fermentations by these organisms usually cause bloater damage in the partially-cured brined cucumbers.

The pHs of various sizes of fresh, pickling cucumbers, ranging from ¾ to 2 in. dia., are shown in Table VII. The pH range listed is favourable for good initial growth of lactic acid bacteria in brined fruit and the pH drops quickly as the acid-forming bacteria become established. Buffering capacity of the green fruit is important because it influences the extent of sugar utilization by lactic acid bacteria. Smaller sizes of cucumbers possess more buffering action than larger sizes; also, a tighter pack of cucumbers in the tank increases the buffering capacity of the brine (Etchells and Moore, 1971).

Nutrients available for the fermentation must diffuse through the tissue and skin of the fruit into the surrounding brine. In most fermentations, however, nutrients are present in the brine in sufficient concentrations within 24 h for growth of *L. plantarum* (Costilow and Fabian, 1953).

Although smaller sizes of cucumbers (up to 1¼ in. dia.) were in most demand until recent years, and still command a premium price to the grower, the trend now is toward the harvest of larger sizes (1½-2 in. dia.) of the fruit. An increased demand for hamburger dill slices, dill spears, and pickle relishes has likewise increased the demand for larger sizes of cucumbers. This demand is fortunate in one respect because larger sizes are more adaptable to mechanical harvesting. On the other hand, larger sizes, which are more susceptible to bloater damage, are difficult to brine properly. This is probably due to structural changes in the fruit as it increases in size and nears ripening (Fleming *et al.*, 1973a). Our recent brining work has been directed toward developing a procedure which will provide superior brine-stock pickles of all sizes, particularly the larger ones.

Until recently, little attention has been paid to the gaseous metabolic activities of cucumbers after they are brined. Brined cucumbers continue to produce carbon dioxide which remains dissolved in the brine prior to the onset of bacterial fermentation (Fig. 8). This amount of carbon dioxide plus the small quantity respired by *L. plantarum* is sufficient to cause bloater damage under average controlled conditions (Etchells and Hontz, unpublished; Fleming *et al.*, 1973a).

TABLE VI

Acid production by *L. plantarum*, *P. cerevisiae* and a combination of the two species in brined cucumbers[1]

Culture	Brine acidity as lactic (%) after the following incubation time in days				Final pH
	0.75	3	10	36	
L. plantarum	0.19	0.45	0.80	1.04	3.3
P. cerevisiae	0.24	0.43	0.50	0.53	3.7
L. plantarum + *P. cerevisiae*	0.18	0.43	0.48	1.06	3.4

[1] Values are averages of two fermentations per culture treatment, calculated from the data described in the pure culture patent by Etchells *et al.* (1968a).

TABLE VII

The pH of fresh, pickling cucumbers of different sizes (Model variety)[1]

Diameter (in.)	Weight (g)	pH of blended tissue
⅜—⅝	5 to 10	6.1
⅝—⅞	10 to 25	5.9
⅞—1⅛	25 to 50	5.8
1⅛—1⅜	50 to 75	5.7
1⅜—1½	75 to 100	5.8
1½—1¾	100 to 150	5.7
1¾—1⅞	150 to 200	5.8
1⅞—2 2[1]	200 to 300	5.2
> 2	> 300	4.4 to 5.0

[1] Data of Bell (1951).

[2] Ripe or near ripe cucumbers may be pH 3.8 or even lower.

The advent of mechanization in harvesting, grading, and handling of cucumbers prior to brining; and, the extended holding periods due to long-distance transportation, have, in some instances caused serious quality deterioration of the brine-stock pickles. Brining of the cucumbers with microbial surface growth, especially of the higher fungi, on the raw fruit can cause a reduction of texture which may not be obvious until after the stock is removed from brine storage. Another serious problem is caused by in-

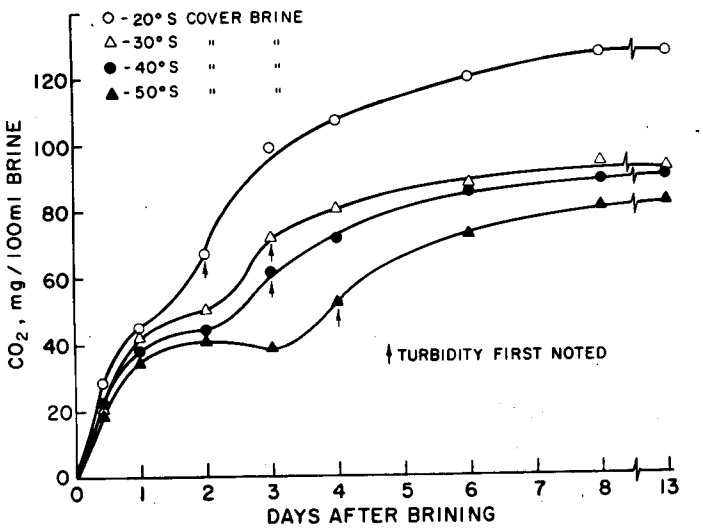

Fig. 8. Carbon dioxide accumulation in cucumber brines undergoing fermentation by natural microflora. From Fleming *et al.* (1973b).

jury of the fruit during harvesting and handling. *Such injured fruit bloats more easily near the injured region.*

Pure Culture Fermentation Studies

The technical foundation for the long-sought 'Controlled Bulk Fermentation' probably was the findings described for the performance of various pure culture, lactic acid bacteria (Etchells *et al.*, 1964). This work led to the development of 'In-Container' or 'Ready to Eat' products such as: dill pickles; dill tomatoes; various types of peppers; dill okra; dill carrots (whole or dill strips); dill green beans; and the like. The detailed processes for these and related products are described in a public service patent (Etchells *et al.*, 1968a). These studies showed that heat-shocking and aseptic packing of the product into sanitized containers, followed by covering with a heated (170° F) and cooled (40° F) brine and inoculation with pure cultures of lactic acid bacteria, produced controlled fermentation. The process has been used to study fermentation properties of bacteria in pure culture and to evaluate the brining qualities of new cucumber varieties. Many problems were encountered, however, in attempting to adapt this 'pure-culture' principle to the large tanks (containing 5000-30,000 lb) used commercially for bulk fermentation of cucumbers. Even so, a bulk fermentation procedure has been proposed (Etchells *et al.*, 1973b).

Our interest in green olives originated from our desire to test the pure-culture process for 'In-Container' or 'Ready to Eat' products for another commodity—Spanish-type, fermented green olives (Etchells *et al.*, 1969). The olive fruit, Manzanillo variety, received the conventional lye treatment to remove most of the bitterness (yet leave sufficient fermentables) and then was washed to remove the lye. Next, the olives were heated in water at 74° C (165° F) for 3 min and then covered with cool (40° F) 40° salometer brine so as to equalize at about 30°C (86° F) prior to inoculation with *L. plantarum*.

The striking effect of the heat shock treatment on the fermentation behaviour of Manzanillo olives by *L. plantarum* is shown in Fig. 9. The marked increase in both the amount and rate of acid production in the brines is clearly

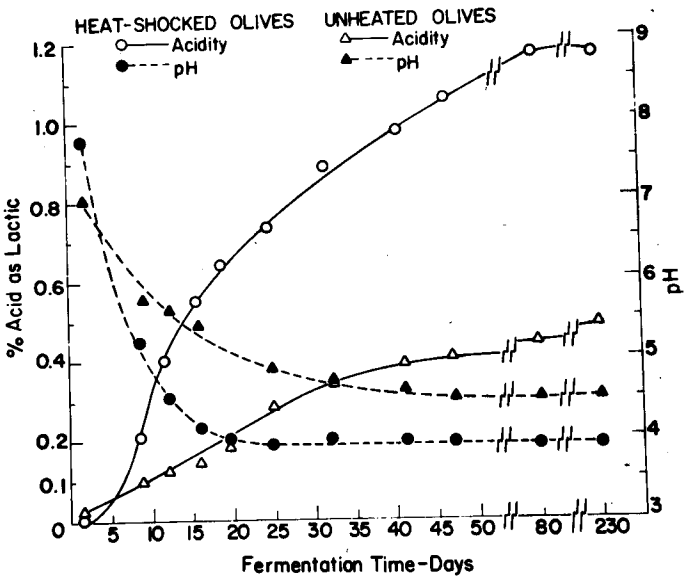

Fig. 9. Effect of heat-shocking Manzanillo olives on their subsequent acid fermentation by *Lactobacillus plantarum*. From Etchells *et al.* (1966).

evident (Etchells *et al.*, 1966, 1969). These observations prompted our inquiry into why heat rendered green olives more fermentable by lactic acid bacteria, a phenomenon that we have not observed with cucumbers or other fruits and vegetables with which we have experimented.

Alkali destroys oleuropein, the bitter phenolic glucoside in green olives. Olives that were neither alkali treated nor heated did not develop an acid fermentation when the brines were inoculated with various species of lactic acid bacteria; only yeasts developed in these brines (Etchells *et al.*, 1966). On the other hand, heating rendered the olives fermentable by lactic acid bacteria whether they were alkali treated prior to brining or not. It has since been found that certain varieties of green olives contain a compound which inhibits growth of *L. plantarum, L. brevis, P. cerevisiae,* and *Leu. mesenteroides,* listed in approximate order of their increasing sensitivity (Fleming and Etchells, 1967). The isolated compound was a phenol which was devoid of acid hydrolyzable reducing sugar; it was suggested that the compound may be the alygcone of oleuropein (Fleming *et al.*, 1969). Further work has shown that the inhibitory compound *is not* preformed in the fresh green olives, but is formed *after* the olives are brined. It was thought that oleuropein, which was not found to be appreciably inhibitory itself, is degraded to the inhibitory compound(s) (Fleming *et al.*, 1969). We have since found that the hydrolysis products of oleuropein, elenolic acid and the aglycone of oleuropein are inhibitory; B-3, 4-dihydroxyphenylethyl alcohol, like oleuropein, did not inhibit growth of lactic acid bacteria (Walter *et al., 1973; Fleming et al.*, 1973c).

Controlled Fermentation of Cucumbers Brined in Bulk

The flow sheet (Fig. 10) outlines and briefly describes the important steps taken to overcome the complexity of variables involved in the control of bulk fermentation. It was deemed impractical to rid the fruit of contaminating micro-organisms with the use of heat as in the above pure-culture process. Rather, thorough washing of the cucum-

bers, followed by in-container chlorination, has been used to reduce the initial microbial load. Acidification of the cover brine effectively suppresses growth of the coliform bacteria and other undesired related types during the initial equilibration of the cucumbers and brine.

Since the temperature of the incoming cucumbers may vary, a formula was developed whereby picklers may calculate and adjust the temperature of the cover brine so that the desired equilibrated cucumber-brine temperature of 80-85° F may be achieved (Etchells and Hontz, 1972; Etchells *et al.*, 1973b).

After equilibration with the initial salt, but prior to the second salt addition, and about three to four hours before the addition of the starter culture, sodium acetate is added to buffer the cover brine. This is to insure that all of the fermentable sugars will be utilized during the active stage of growth of the lactic starter culture. Otherwise, subsurface yeasts could later ferment this sugar and produce undesirable levels of carbon dioxide, sufficient to cause bloater damage.

It is desired that the lactic culture(s), used as the inoculum, provide a rapid and complete conversion of fermentable sugars to lactic acid, before the small number of contaminating micro-organisms can become established in the brine and produce undesirable end-products. The added culture(s) should give high fermentative performance under the conditions in the brine, 24-30° C (75-85° F, 6-8% NaCl), and should produce a minimum amount of carbon dioxide. The culture(s) should have desirable preservation and activation properties. High-performance cultures of *P. cerevisiae* and *L. plantarum* in combination or *L. plantarum* alone, have been sucessfully used. The strain(s) of *P. cerevisiae* used should not inhibit *L. plantarum*.

1. THE CUCUMBERS:	Examine green-stock carefully as to quality; grade out stock that is diseased, broken or mouldy.
2. WASHING:	Wash graded stock thoroughly with a brush- or reel-type washer. Temper refrigerated stock with warm water.
3. IN-TANK SHRINKING:	Shrinking of stock may be necessary by allowing cushion brine to reduce the cucumber-mass to the desired head level, with ratio of at least 60% cucumbers and 40% brine by wt. Unwashed stock flumed in brine to tanks may need no further shrinkage. But should be washed in the tank.
4. COVERING AND HEADING:	Cucumbers are covered at the desired volume level with a 'false head' made of wooden boards and keyed down securely with heavier timbers of appropriate length. The head boards should provide plenty of avenues for fermentation and purging gases to escape.
5. COVER BRINE AND CHLORINE:	Add a chlorinated (about 50 ppm) 25° salometer (6.6% NaCl) brine until the liquid level is 4-6 in. above the head boards and about 4 in. below the top of the tank.
6. ACIDIFICATION:	The chlorinated cover brine must be carefully acidified with acetic acid or its equivalent amount of 200 grain vinegar. The acetic acid is added at the rate of 6 ml/gal of total cucumbers and brine. Chlorinate again, about 12 h after adding cover brine.
7. SALT ADDITIONS:	Salt is added on the 'false head' to maintain the initial brine strength, which otherwise would be diluted by the water content of the cucumbers (approx. 95%). For the 25° salometer treatment, 6-lb of salt is needed for each cwt. (100 lb) of stock brined. The amount of salt added at the outset, and after one or two days, will depend on the size brined. Large stock will require 2-3 additions.
8. ACETATE ADDITIVE:	Na acetate ($CH_3COONa.3H_20$) is added 2-3 h before the second addition of salt, at the rate of 0.5% wt. (= 18.8 g per gallon of packed and brined material with brine calculated as H_2O as to wt.)/7.6 lb./1,000 lbs. cucumbers brined.
9. CULTURE ADDITION:	The bulk-brined cucumbers are usually inoculated 18-24 h *after* the initial brining operation and at least 2-3 h *after* the acetate addition. Inoculation is usually made with two species of lactic acid bacteria; *P. cerevisiae* and *L. plantarum*; or the latter species alone, at the rate of 4 million cells/gal. brined material.
10. NITROGEN GAS PURGING ACTION:	N_2 purging is begun as soon as the tank is headed, brined and acidified. The type and amount or rate of purging will depend on schedules for various cucumber sizes and capacity of container.

Fig. 10. Flow sheet for the controlled fermentation of cucumbers brined in bulk (25° salometer treatment; 6.6% NaCl/wt). For full details that apply to this procedure and related studies on brining, see Etchells, J. L. and Hontz, L. H. (1972) and especially, Etchells, J. L., Bell, T.A., Fleming, H.P., Kelling, R.E. and Thompson, R. L. (1973b).

Nitrogen purging of the brine is used to reduce undesirable levels of carbon dioxide that are produced. We did not expect that bloating would be a problem with pure-culture fermentations by the homofermentative species, *L. plantarum*. Pasteurized cucumbers inoculated with this bacterium (Etchells *et al.*, 1968b) did not bloat although fermentation by *L. brevis*, a high producer of carbon dioxide, did cause bloating. It was quite startling, to say the least, then to find that with unheated, large-sized cucumbers, serious bloating occurred even when *L. plantarum* was the fermenting species (Etchells, Bell, Fleming, Kelling and Thompson, 1973b)! We found that when unheated cucumbers are brined, enough carbon dioxide is liberated by respiration of the fruit into the brine (Fig. 8) which, when combined with the small quantity produced by *L. plantarum*, is sufficient to cause bloater damage (Fleming *et al.*, 1973a)! As bloater development occurs, formation of gas pockets inside the cucumbers causes liquid to be expressed with a resultant rise in the level of the cover brine. This rise in brine level, termed 'expansion volume', was used as an index of bloater development. Removal of carbon dioxide during the fermentation by nitrogen purging caused a decrease in the expansion volume (Fig. 11). It has been found repeatedly that restricting a build-up of carbon dioxide in the brine during the entire fermentation, using the controlled process (Fig. 10), prevents bloater formation.

We have consistently obtained superior quality brine-stock when the suggested brining process (Fig. 10) has been used. The active fermentation is usually completed within 10 to 12 days, often less, depending on the brine temperature, with no residual sugar (Fig. 12). No problems have been encountered with coliform bacteria, heterofermentative lactic acid bacteria, or subsurface yeasts which may be present on the fruit even after the washing and

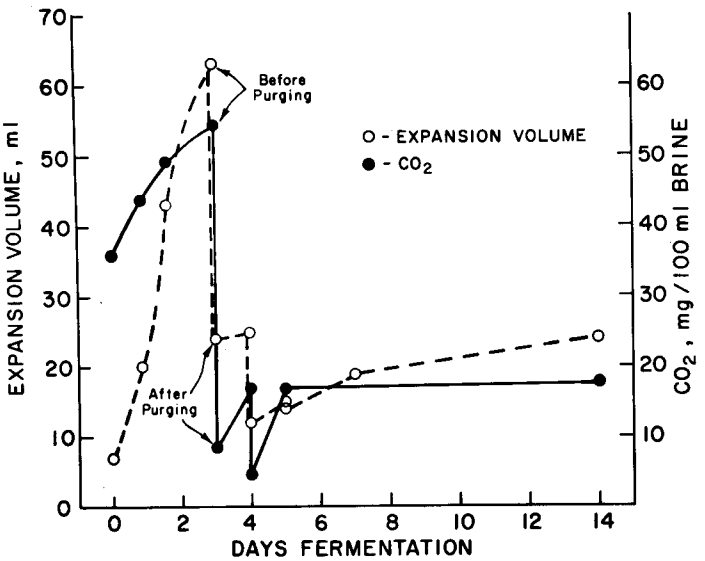

Fig. 11. Effect of nitrogen purging on reducing the carbon dioxide concentration and expansion volume of pure culture fermented cucumbers. From Fleming *et al.* (1973a).

chlorination treatments. After the active fermentation is complete, a transparent cover such as a small, plastic, dome should be locked in place over the tank to permit the use of lower brine-holding strengths as well as to prevent contamination by film yeasts, insects, and other foreign matter.

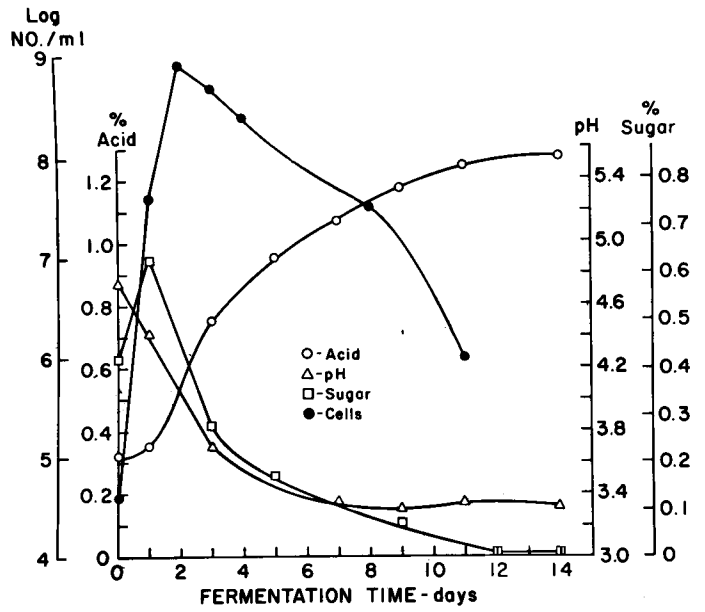

Fig. 12. Controlled fermentation of cucumbers brined in bulk. Equilibrated brine strength during fermentation, 6.4% NaCl; incubation temperature, 27° C.

Summary

Major factors which influence the progress of the lactic acid fermentation of brined cucumbers and certain other vegetables are discussed. These factors, which have been investigated over a period of several decades, include brining and environmental conditions as they affect growth of the various microbial species in natural fermentations. Fermentation by the homofermentative lactic acid bacteria is desired. The growth of yeasts, coliform bacteria and heterofermentative lactic acid bacteria have caused quality defects in the cucumbers such as bloater damage (hollow stock) and offensive odour and taste. Fungal enzymes present on green cucumbers have caused texture deterioration of the product during brine storage. A means of assuring a usually predictable fermentation with consistently superior quality brine-stock pickles is outlined, the necessary details of which are being reported in another publication for the pickle industry. Essentials of the process were based on our previous studies. The suggested process centers around favouring a prompt and vigorous homofermentative lactic acid fermentation by the introduction of starter cultures, coupled with the control of undesirable microbial activity and fermentation gases.

Acknowledgements

The material included herein for this symposium is the result of efforts of many persons who have cooperated in our work; some of these persons are cited as co-authors in the references. We regret we cannot cite everyone, but we are nonetheless most grateful for the contributions they have made.

We also are grateful to Pickle Packers International, Inc., St. Charles, Illinois 60174, USA, and especially to W. R. Moore, Jr., Executive Vice President of that organization, for continuing encouragement and financial assistance over the years in conducting the research. Numerous pickling companies and their personnel have made notable contributions to our cooperative research program; they have not only provided direct assistance, but also needed supplies, raw materials, and brining facilities.

We also are indebted to many scientists in other disciplines at North Carolina State University and the U.S. Department of Agriculture, who have provided needed assistance.

References

Bell, T. A. (1951). Pectolytic enzyme activity in various parts of the cucumber plant and fruit. *Bot. Gaz.* 113, 216-221.

Bell, T.A. and Etchells, J. L. (1958). Pectinase inhibitor in grape leaves. *Bot. Gaz.* 119, 192-196.

Bell, T.A., Etchells, J. L. and Costilow, R. N. (1958). Softening enzyme activity of cucumbers from northern brining areas. *Fd Res.* 23, 198-204.

Bell, T. A., Etchells, J.L., Williams, C.F. and Porter, W. L. (1962). Inhibition of pectinase and cellulase by certain plants. *Bot. Gaz.* 123, 220-223.

Bell, T.A., Etchells, J.L., Singleton, J. L. and Smart, W. W. G., Jr. (1965a). Inhibition of pectinolytic and cellulolytic enzymes in cucumber fermentations by sericea. *J. Fd Sci.* 30, 233-239.

Bell, T. A., Etchells, J. L. and Smart, W. W. G., Jr. (1965b). Pectinase and cellulase enzyme inhibitor from sericea and certain other plants. *Bot. Gaz.* 126, 40-45.

Bell, T. A., Etchells, J. L. and Kelling, R. E. (1971). A quick test for cucumber brine acidity. *J. Fd Sci.* 36, 1036-1038.

Costilow, R. N. and Fabian, F. W. (1953). Availability of essential vitamins and amino acids for *Lactobacillus plantarum* in cucumber fermentations. *Appl. Microbiol.* 1, 320-326.

Costilow, R. N., Coughlin, F. M., Robach, D. L. and Ragheb, H. S. (1956). A study of the acid-forming bacteria from cucumber fermentations in Michigan. *Fd Res.* 21, 27-33.

Etchells, J. L. (1938). Rate of heat penetration during the pasteurization of cucumber pickle. *Fruit Prod. J.* 18, 68-70.

Etchells, J. L. and Bell, T. A. (1950). Film yeasts on commercial cucumber brines. *Fd Technol.* 4, 77-83.

Etchells, J. L. and Jones, I. D. (1942). Pasteurization of pickle products. *Fruit Prod. J.* 21, 330-332.

Etchells, J. L. and Jones, I. D. (1943). Bacteriological changes in cucumber fermentation. *Fd. Inds.* 15, 54-56.

Etchells, J. L. and Jones, I. D. (1946). Characteristics of lactic acid bacteria from commercial cucumber fermentations. *J. Bact.* 52, 593-599.

Etchells, J. L. and Moore, W. R., Jr. (1971). Factors influencing the brining of pickling cucumbers —questions and answers. *Pickle Pak Sci.* 1, 3-17.

Etchells, J. L., Costilow, R. N. and Bell, T. A. (1952). Identification of yeasts from commercial cucumber fermentations in northern brining areas. *Farlowia* 4, 249-264.

Etchells, J. L., Bell, T. A., Monroe, R. J., Masley, P. M. and Demain, A. L. (1958). Populations of softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. *Appl. Microbiol.* 6, 427-440.

Etchells, J. L., Borg, A. F. and Bell, T. A. (1961a). Influence of sorbic acid on populations and species of yeasts occurring in cucumber fermentations. *Appl. Microbiol.* 9, 139-144.

Etchells, J. L., Costilow, R. N., Bell, T. A. and Rutherford, H. A. (1961b). Influence of gamma radiation on the microflora of cucumber fruit and blossoms. *Appl. Microbiol.* 9, 145-149.

Etchells, J. L., Costilow, R. N., Anderson, T. E. and Bell, T. A. (1964). Pure culture fermentation of brined cucumbers. *Appl. Microbiol.* 12, 523-535.

Etchells, J. L., Borg, A. F., Kittel, I. D., Bell, T. A. and Fleming, H. P. (1966). Pure culture fermentation of green olives, *Appl. Microbiol.* 14, 1027-1041.

Etchells, J. L., Bell, T. A. and Costilow, R. N. (1968a). Pure culture fermentation process for pickled cucumbers. U. S. Patent No. 3,403,032.

Etchells, J. L., Borg, A. F. and Bell, T. A. (1968b). Bloater formation by gas-forming lactic acid bacteria in cucumber fermentations. *Appl. Microbiol.* 16, 1029-1035.

Etchells, J. L., Bell, T. A. and Kittel, I. D. (1969). Pure culture fermentation of green olives. U. S. Patent No. 3,480,448.

Etchells, J. L. and Hontz, L. H. (1972). Suggestions for brining pickling cucumbers—the use of equilibrated brine strengths, based on the average brine-cucumber-mass temperature. *Pickle Pak Sci.* 2, 18-19.

Etchells, J. L., Bell, T. A., Costilow, R. N., Hood, C. E. and Anderson, T. E. (1973a). Influence of temperature and humidity on microbial, enzymatic, and physical changes of stored, pickling cucumbers. *Appl. Microbiol.* 26, 943-950.

Etchells, J. L., Bell, T. A., Fleming, H. P., Kelling, R. E. and Thompson, R. L. (1973b). Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers—the use of starter cultures and reduction of carbon dioxide accumulation. *Pickle Pak Sci.* 3, 4-14.

Fleming, H. P. and Etchells, J. L. (1967). Occurrence of an inhibitor of lactic acid bacteria in green olives. *Appl. Microbiol.* 15, 1178-1184.

Fleming, H. P. and Etchells, J. L. (1968). *Bacteriol. Proc.* p. 7. (Abstr.)

Fleming, H. P., Walter, W. M., Jr. and Etchells, J. L. (1969). Isolation of a bacterial inhibitor from green olives. *Appl. Microbiol.* 18, 856-860.

Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E. and Bell, T. A. (1973a). Bloater formation in brined cucumbers fermented by *Lactobacillus plantarum*. *J. Fd Sci.* 38, 499-503.

Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E. and Bell, T. A. (1973b). Carbon dioxide production in the fermentation of brined cucumbers. *J. Fd Sci.* 38, 504-506.

Fleming, H. P., Walter, W. M., Jr. and Etchells, J. L. (1973c). Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Appl. Microbiol.* 26, 777-782.

Jones, I. D. and Etchells, J. L. (1943). Physical and chemical changes in cucumber fermentations. *Fd Inds* 15, 62-64.

Jones, I. D., Etchells, J. L., Veldhuis, M. K. and Veerhoff, O. (1941). Pasteurization of genuine dill pickles. *Fruit Prod. J.* 20, 304-5, 316, 325.

Mrak, E. M. and Bonar L. (1939). Film yeasts from pickle brines. *Zentbl. Bakt. ParasitenK. II.* 100, 289.

Pederson, C. S. and Albury, M. N. (1950). Effect of temperature upon bacteriological and chemical changes in fermenting cucumbers. *N.Y. St. Agric. Expt. Sta. Bull. No. 744.*

Pederson, C. S. and Albury, M. N. (1956). Variations in bacterial flora of low salt cucumber brines. *Appl. Microbiol.* 4, 259-263.

Pederson, C. S. and Albury, M. N. (1961). The effect of pure culture inoculation on fermentation of cucumbers. *Fd Technol.* 15, 351-354.

Pederson, C. S. and Ward, L. (1949). The effect of salt upon the bacteriological and chemical changes in fermenting cucumbers. *N.Y. St. Agric. Expt. Sta. Tech. Bull. No. 288.*

Rosen, S. and Fabian, F. W. (1953). The importance of biotin, niacin, and pantothenic acid in cucumber fermentation. *Fd Technol.* 7, 244-247.

Walter, W. M., Jr., Fleming, H. P. and Etchells, J. L. (1973). Preparation of antimicrobial compounds by hydrolysis of oleuropein from green olives, *Appl. Microbiol.* 26, 773-776.

Note: *Paper No. 4207, Journal Series, North Carolina Agricultural Experiment Station, Raleigh.

"The paper reprinted herein was prepared for, and presented to, the Fourth Long Ashton Symposium, the theme being, "Lactic Acid Bacteria in Beverages and Food." The Symposium was held September 17-21, 1973, University of Bristol, Research Station, Long Ashton, Bristol, England BS18 9AF, United Kingdom.

The article is reprinted by permission of the original publisher, Academic Press (London) Limited, and by permission of the authors."