DEVELOPMENT OF A SPICE FORMULATION FOR PURE CULTURED FERMENTED CUCUMBER PICKLES

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ABSTRACT

DEVELOPMENT OF A SPICE FORMULATION
FOR PURE CULTURE FERMENTED
CUCUMBER PICKLES

By

Cecelia Kuhnley Marshall

The objective of this study was to develop a pure culture fermented pickle with good flavor that did not have the spice formulation rendered unacceptable by the fermentation process. Nine different spice formulations were evaluated for this purpose.

Various chemical analyses were used to monitor the course of fermentation of cucumbers by pure cultures of three strains of Lactobacillus plantarum, two strains of Pediococcus cerevisiae and mixtures of both. Total plate counts were used to follow the growth of the organisms and to determine the effects of various spice formulations on the cultures. The different spice formulations were evaluated for flavor acceptability, and the best formulation was submitted to taste panels. Attempts were made to determine the effects of the various microorganisms on a spice formulation

by gas-liquid chromatographic analysis of the headspace in sample jars.

A spice formulation containing oil of dill weed, oleoresin-oil of garlic, oleoresin capsicum, caraway oil, pimenta leaf oil, and polysorbate 80, when added at a level of 0.94% (v/v) to a brine containing 6.7% (w/w) salt, was found to produce a good product. Differences in the flavor of the final product were noted, depending on which microorganism was responsible for the fermentation. Thus, the flavor of products fermented by various strains of Lactobacillus plantarum and Pediococcus cerevisiae were evaluated subjectively and objectively.

Subjective taste panel evaluations indicated that the pediococci produced a bland pickle, lactobacilli produced sour pickles (the degree of sourness depended on the level of acid produced), and the mixed cultures of P. cerevisiae and L. plantarum tended to counterbalance each other by accentuating certain flavor characteristics and attenuating others. Hedonic ratings by taste panels indicated that preference in pickles varied with individual tastes and that, overall, no one sample was preferred above another.

Gas-liquid chromatography, using flame ionization and electron capture detectors, failed to show any differences in the headspace profiles of the various samples. However, addition of

cucumbers to the brine, whether or not they were fermented, resulted in an unexplained disappearance of two peaks which appeared in the chromatograms of the brine. Although the differences in flavor produced by the fermenting microorganisms were evident by organoleptic evaluation, they were not detected by this chromatographic system. The variation in flavor was the result of some change in trace volatile constituents or in the non-volatile flavor constituents.

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INTRODUCTION

The pickling industry incurs large economic losses annually due to defective salt stock produced during the natural fermentation of cucumbers. Bloaters or hollow pickles may be produced as a result of a gaseous fermentation by coliforms, yeasts, and/or heterofermentative lactobacilli. Softening of pickles is the result of the action of pectin-splitting enzymes produced by molds growing on the cucumber blossoms. Mechanical harvesting increases the number of blossoms that remain on the cucumbers, allowing enzymatic action to occur during fermentation. Several treatments have been proposed to control the natural fermentation, but a consistent quality salt stock has not been achieved.

Etchells, Bell, and Costilow (1968a) patented a process for a completely controlled pure culture fermentation. The advantages of such a process include reduced pickling time, consistent high quality products, reduction of losses due to spoilage and defects, suitability of consumer-sized containers for the "in jar" fermentation, and lower labor costs since the produce must be processed only once.

However, there have been flavor problems with pure culture pickles (Dr. R. N. Costilow, personal communication). The flavor produced by pure culture fermentation in the absence of spices is not acceptable. When spices have been added to the covering brines, the fermented products have had different flavors, depending on which microorganism was responsible for the fermentation. The objective of this study was to develop a spice formulation suitable for commercial production of a pure culture fermented pickle. An attempt was also made to objectively evaluate, by GLC headspace analysis, the differences in flavor of the final products as a result of the fermentation by strains of Lactobacillus plantarum and Pediococcus cerevisiae.

REVIEW OF LITERATURE

The Natural Fermentation of Cucumbers

Extensive research on factors involved in the natural fermentation of cucumbers and other vegetables has been done since the early nineteen-thirties, much of it by the U. S. Food Fermentation Laboratory and the North Carolina Agricultural Experiment Station, North Carolina State University, Raleigh, North Carolina.

The process of brining cucumbers for natural fermentation has been reviewed elsewhere (Etchells and Jones, 1946; Etchells, Jones, and Bell, 1951c; Cruess, 1958; Binsted, Devey, and Dakin, 1962). There is an initial loss of water and water-soluble nutrients from cucumbers after brining (Jones and Etchells, 1943; Jones et al., 1940). The cucumber tissue becomes more permeable, allowing sugar and other organic substances to leave the cells and salt and acid to enter (Jones and Etchells, 1943; Pederson and Albury, 1962). Salt brining exerts a selective action on the microorganisms indigenous to the cucumber, thus, the resulting fermentation will lead to the production of acid. The combined salinity and acidity of the product are responsible for its preservation.

Etchells and Jones (1943) have reported that three groups of microorganisms may be involved in the natural fermentation. Species of Aerobacter are salt-tolerant and may begin the fermentation with the production of equal amounts of hydrogen and carbon dioxide. The lactic acid bacteria are responsible for producing the desired level of acid. These organisms may be homofermentative, in which case lactic acid accounts for 90 to 95% of the end products of fermentation, small amounts of acetic and formic acids and carbon dioxide also being produced (Etchells and Jones, 1946; Christensen, Albury, and Pederson, 1958). The heterofermentative bacteria, e.g. Lactobacillus brevis, produce copious amounts of gas and acetic, lactic, and formic acids in decreasing amounts (Christensen et al., 1958). Two groups of yeasts, fermentative and oxidative, may be involved in the natural fermentation of brined cucumbers (Etchells and Jones, 1951a). Those which produce an alcohol and gaseous fermentation in the brine include, primarily, Torulopsis holmii and Brettanomyces versatilis, as well as other species of these genera and of Hansenula, Torulaspora, and Zygosaccharomyces. Debaryomyces membranaefaciens was found to be the most prevalent film yeast; others include species of Endomycopsis and Zygosaccharomyces and Candida krusei (Etchells and Jones, 1951a; Costilow and Fabian, 1953). The film yeasts grow luxuriously on

the tops of fermentation vats and can utilize the lactic acid as a source of carbohydrate, thus raising the pH of the brine and allowing the growth of other undesirable spoilage microorganisms. The course of fermentation is directed, at least in part, by the initial inoculum provided by the cucumber (Etchells and Jones, 1943).

The production of lactic acid by the homofermentative bacteria, i.e., Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae, and Lactobacillus plantarum, in the order of increasing prevalence (Pederson and Ward, 1949; Pederson and Albury, 1950; Costilow and Fabian, 1953; Costilow et al., 1956), is most desirable, resulting in preservation of the pickles. The gaseous fermentation by coliforms, heterofermentative lactic acid bacteria, and yeasts is considered to be a competitive and undesirable fermentation (Etchells and Jones, 1943, 1951a; Etchells et al., 1951c; Etchells, Borg, and Bell, 1968b), because "bloaters" or hollow pickles result. Bloaters reduce the value of the cured stock since they must be used for cut pickle or relish items, and represent a tremendous loss of profit to the pickle industry annually (Etchells et al., 1951c).

Many attempts have been made to control the natural fermentation to reduce the incidence of gas production and bloater formation. The salt-tolerant organisms, including species of

Aerobacter, which can initiate the fermentation with the production of gas, are very sensitive to acid (Etchells and Jones, 1951a). Thus, it is important that there be rapid and immediate growth of the lactic acid bacteria after brining the cucumbers. Jones et al. (1940) studied the effects of initial salt concentration and the addition of acid or sugar to the brines. They concluded, as have others (Etchells and Jones, 1946; Pederson and Ward, 1949; Costilow and Fabian, 1953) that brines of lower salt concentrations (10% or less) result in higher acid production. The acid forming bacteria seem to be inhibited by higher salt concentrations (Jones et al., 1940; Etchells and Jones, 1943; Pederson and Ward, 1949; Etchells and Jones, 1951a; Etchells et al., 1951c), and the retardation of their growth allows the more salt-tolerant Aerobacter species to proliferate. The addition of sugar to the brine has been found to increase the populations of lactic acid bacteria, but it also results in the increased growth of gas and alcohol-producing yeasts, with a subsequent increase in the numbers of bloaters (Jones et al., 1940; Etchells et al., 1951a). Addition of acetic acid (vinegar) to cucumber brines delayed acid production and encouraged yeast fermentation; when lactic acid was added, the acid-forming bacteria were markedly inhibited, and an active and prolonged yeast fermentation resulted in the formation of bloaters (Jones et al., 1940). The temperature of

the brine, which is usually 80 to 86 F, does not appear to affect the fermentation to any great extent (Etchells and Jones, 1946), since the optimum temperature for the lactic acid bacteria, both gas-producers and non-gas-producers, is between 75 and 86 F (Pederson and Albury, 1950). Lower temperatures seem to favor growth of Leuconostoc species (Etchells and Jones, 1946; Pederson and Albury, 1950).

The control of film yeasts is easily accomplished by ultraviolet light, as in direct sunlight (Rahn, 1931; Binsted et al., 1962), but this is not a suitable means of control since the fermentation vats are located outdoors where gross contamination by dust, insects, rodents, and birds and dilution of the brine by rain can occur. Mustard oil, neutral mineral oil, or liquid paraffin could be floated on the surface of the vats in a thin layer (Blum and Fabian, 1943; Cruess, 1958; Binsted et al., 1962), but it is difficult to remove the last traces of these from the cucumbers after fermentation. The volatility of mustard oil also precludes its use.

Phillips and Mundt (1950) suggested using sorbic acid as a means of preventing the growth of scum yeasts, because it is nontoxic to humans and has no flavor or odor. Sorbic acid (2, 4-hexadienoic acid) appears to selectively inhibit all catalase positive bacteria, yeasts, and molds by suppression of fumarate oxidation

(York and Vaughn, 1955). The inhibition by sorbic acid depends on the concentration of undissociated acid, which is greatest at pH 5.0 or less (Costilow, Ferguson, and Ray, 1955; Etchells, Bell, and Borg, 1955). Levels of 0.1% sorbic acid were found to effectively depress the growth of both fermentative and oxidative yeasts (Phillips and Mundt, 1950; Borg, Etchells, and Bell, 1955; Costilow et al., 1955); but some species of Candida, particularly C. krusei, were still present in the sorbic acid treated brines (Borg et al., 1955; Costilow et al., 1955; Etchells, Borg, and Bell, 1961). Phillips and Mundt (1950) and Borg et al. (1955) reported reduced populations of acid-forming bacteria and a subsequently slower acid development in brines to which 0.1% sorbic acid had been added. Experiments by Costilow et al. (1957b) showed that the adverse effect of sorbic acid on the acid fermentation increased with salt concentration, and that a level of 0.03% sorbic acid would still inhibit yeasts without having an effect on the acid fermentation. However, Borg et al. (1955) and Costilow (1957a) reported that all levels of sorbic acid slowed the rate and quality of the cure, and yielded pickles with inferior color. Costilow et al. (1956) found that L. plantarum was slightly less active than L. brevis in sorbic acid treated brines. Etchells, Borg, and Bell (1968b) have confirmed that bloaters still occur in sorbic acid treated brines as a result of the gaseous fermentation by L. brevis.

Softening of pickle stock has also been a serious problem to the pickle industry. This problem has been thoroughly reviewed by Bell, Etchells, and Jones (1950); Etchells and Jones (1951b); Cruess (1958); and Binsted et al. (1962).

Pasteurization of unfermented, partially fermented, and fermented genuine dill pickles has been advocated for their preservation (Etchells and Jones, 1942, 1944, 1951b). Heating the product to an internal temperature of 165 F for 15 min, followed by rapid cooling, was found to destroy vegetative microorganisms and to inactivate enzyme systems without adversely affecting the texture and crispness of the pickles. Pasteurization actually improved the texture of genuine dill pickles, as these will soften during storage if not pasteurized (Jones et al., 1941). More recent studies on the influence of various acidities and over- and under-pasteurization of fresh pack pickles have been reported (Monroe et al., 1969; Nicholas and Pflug, 1961). It was hoped that pasteurization would provide a standardized procedure for preserving and controlling the quality of the finished products.

Pederson and Albury (1961) found that the course of the natural fermentation could be controlled to some extent by the addition of certain species of lactic acid bacteria to cucumber brines. However, because the fermentation depends to such a great

extent on the natural flora of the cucumbers, no one brining treatment discussed will consistently give the same quality salt stock from year to year.

Pure Culture Fermentation of Cucumbers

Etchells et al. (1964) studied the feasibility of a pure culture fermentation in the absence of competitive microorganisms. There was some precedence for such experiments when Engelland (1962) patented a process for the pure culture fermentation of shredded cabbage for sauerkraut. The use of a heat shock (66 to 82 C water) was found to be effective in destroying vegetative microorganisms and insured a rapid rate of acid production by the added pure culture. Acidification of the covering brines to pH 5.0 or less with lactic acid (resulting in about 0.10% acidity after equalization) was found to effectively inhibit the growth of sporeforming bacteria, especially anaerobic types, which survived the heat shock. Strains of Pediococcus cerevisiae, Lactobacillus plantarum, and Lactobacillus brevis grew to the highest populations, producing the highest amount of acid and lowest pH values. In mixed cultures, P. cerevisiae grew first, followed by L. plantarum; L. brevis grew at a slower rate and reached lower maximum populations. L. plantarum was found to consistently produce the highest levels of acid; L. brevis was the

lowest acid-producer of the three. Only strains of L. plantarum and P. cerevisiae were found to consistently produce enough acid to insure a brine pH of less than 4.0. L. brevis was also undesirable due to the production of gas, resulting in hollow pickles, or bloaters.

Based on the results of these studies reported in 1964, Etchells, Bell, and Costilow patented the pure culture fermentation process for pickled cucumbers (1968, U.S. Pat. No. 3,403,032). The advantages of such a process as described in the patent are 1) complete control over the fermentation, decreasing losses in brine stock due to bloaters, softening, bleaching and putrefaction; 2) a final product of consistent and predictable high quality free of undesirable changes in flavor and texture; 3) the self-limiting nature of the process (i.e., development of a certain level of acid causes the organisms to die off), which allows pickled cucumbers and other vegetables to be manufactured in retail and wholesale containers; 4) a consistently firm texture in the finished product which is about the same as that of the starting material; and 5) a reduction in fermentation time, as the process is essentially complete within a week.

A major problem with pure culture fermented pickles has been flavor, associated with the spicing and organoleptic acceptance

of these products (Dr. R. N. Costilow, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan, personal communication). Consultation with one of the pickle manufacturers in Michigan has confirmed the above analysis of the problem.

Analysis of Flavor

The course of fermentation of cucumbers either by pure culture or by the natural flora is followed by increases in brine acidity. But the ultimate evaluation of the fermentation is based on the appearance and taste of the final product. A fermented pickle can easily be distinguished from a non-fermented, or fresh pack, pickle, even if the brines of both contain the same amount of salt and acid. Margalith and Schwartz (1970) discussed the effects of microorganisms on the flavor of fermented products. In order to establish the contribution of a particular species involved in a fermentation process to the flavor of the final product, the approximate chemical composition of the flavor components of that product must be known.

Recognizing the need for work on the flavor of pure culture fermented pickles, Aurand et al. (1965) attempted to identify the flavor constituents introduced by the organisms responsible for the

fermentation. Forss et al. (1962) used GLC to isolate and identify the flavor constituents of fresh cucumbers. Nona-2-trans, 6-cis-dienal and hex-2-enal were found to be responsible for the pleasant "green" or "plant-like" flavor of the fresh cucumber, whereas, non-2-enal apparently contributed to the more unpleasant, astringent flavor. Aurand et al. (1965), using GLC to separate and identify the volatile constituents produced during the pure culture fermentation of cucumbers in an unspiced brine, found that formaldehyde, acetaldehyde, acetone, and propionaldehyde are formed during the fermentation by strains of L. plantarum or P. cerevisiae. Butyraldehyde was also produced during pure culture fermentation by L. plantarum, but not by P. cerevisiae. A peak representing ethyl alcohol appeared on the chromatograms, but there was some question as to whether this was a product of fermentation, since the jars and caps were rinsed in 70% ethanol prior to packing. Fleming, Etchells, and Bell (1969) have since analyzed the volatiles produced during the pure culture fermentation of olives in a similar manner.

Use of Gas-Liquid Chromatography for Flavor Research

The classical approach to flavor analysis was to use distillation of the food or other complicated and time-consuming methods (Burr, 1964). With the advent of GLC, Dimick and Corse (1956)

suggested its use as a simple and rapid means of analyzing the volatile constituents of flavor. The principal contributions to flavor are made by aroma and taste. Hornstein and Teranishi (1967) have compiled an excellent review of what is known about the physiology of taste and odor reception. The characteristic properties of food odors are outlined by Weurman and Lunteren (1967). If the sense of smell is deadened in some way, almost all of the flavor of a food disappears. Since the scientific approach to any problem is to identify it by some objective measurement, and since odor is such a vital part of flavor, attempts have been made to identify the volatile compounds which are responsible for the aroma of a food, in order to correlate odor (an objective measurement) with the subjective evaluation of total flavor of the food (Dimick and Corse, 1956; Jennings, 1957; Teranishi et al., 1963; Burr, 1964; Guadagni et al., 1966; Rogers, 1966; Hornstein and Teranishi, 1967).

Gas-liquid chromatography is especially suitable for separating highly volatile compounds. Flame ionization detectors show a high sensitivity to organic compounds, but are insensitive to inorganic gases and water; are capable of high resolutions; and can be used to detect a tremendous range of samples and sample quantities (Mackay, Lang, and Berdick, 1961; Nawar and Fagerson, 1962; Weurman, 1963; Burr, 1964; Nawar, 1966; Ryder, 1966; and

Hornstein and Teranishi, 1967). Rapid, accurate, reproducible, low temperature analyses of small volumes of samples can now be made without destroying the sample by extraction and concentration. Withdrawing a portion of the headspace vapor above a food provides the simplest, fastest, most precise sampling, with little possibility for artifact formation; and, therefore, most closely resembles organoleptic testing (Mackay et al., 1961; Nawar and Fagerson, 1962; Teranishi, Buttery, and Lundin, 1962; Teranishi et al., 1963; Kepner et al., 1964; and Teranishi and Mon, 1968). Some of the early work using headspace sampling (Jennings, 1957; Buttery and Teranishi, 1961; Bassette, Özeris, and Whitnah, 1962; and Teranishi, Buttery, and Mon, 1964) led to the suggestion that GLC profiles might be used in quality control to follow aroma quality by the appearance or disappearance of certain peaks.

The volatile sulfides in garlic (Oaks, Hartmann, and Dimick, 1964), potatoes (Gumbmann and Burr, 1964), beer (Brenner, Owades, and Fazio, 1955), cabbage (Bailey et al., 1961), and onions (Carson and Wong, 1961) have been analyzed by GLC. Several other foods have also been characterized by their aroma profiles: milk (Bassette et al., 1963), raspberries (Weurman, 1961), orange juices (Wolford et al., 1963), cocoa beans (Bailey et al., 1962), beer (Kloot, Tenny, and Bavisotto, 1958), and sauerkraut (Vorbeck et al., 1961).

There are, however, certain disadvantages to the use of GLC for flavor analyses. Even the most sensitive flame ionization detectors, which can respond to as low a concentration as 10 μg/kg (ppb) (Kendall and Neilson, 1964), are not as sensitive as the human nose (Teranishi et al., 1963, 1964; Bayer, 1966; Weurman and Lunteren, 1967; Hornstein and Teranishi, 1967). The gas chromatograph can adequately detect only compounds with a fairly high odor threshold, the theoretical limit of detection being about that for n-amyl acetate--0.05 μg per liter in air (Teranishi, 1970). Other highly odorous compounds, such as dimethyl sulfide, n-decanal, methyl mercaptan, and β-ionone, can be detected in much lower quantities by human sensors (Guadagni, 1963). These compounds, present below the threshold of the GLC detector, may comprise a mixture which is very characteristic of the flavor of the food product (Elberg, 1967). There is often no correlation between the magnitude of the peak and the contribution of that component to the total flavor or aroma; i.e., the fraction with the most intense odor, or containing a component which is present in the largest quantity, may give a very small peak (Burr, 1964; Sethi, Nigam, and Rao, 1965; Bayer, 1966; and Guadagni et al., 1966). Trace compounds may not appear as a visible peak or could be hidden by a larger peak of a major component with the same retention time (Merritt and Walsh, 1962; Bayer,

1966; Elberg, 1967). The flavor of a food depends not only on which volatiles are present, but also on the concentration and relative ratios of these compounds in the food (Nawar, 1966; Weurman and Lunteren, 1967).

For these reasons, some scientists (Weurman, 1963; Nawar, 1966; Ryder, 1966) feel that direct vapor or headspace analysis gives an oversimplified picture of the total flavor. The ratio of volatiles in the headspace vapor may or may not be the same as that in which they appear in the food; low threshold compounds may not be detected; and only compounds with low boiling points will be characterized. The detection of low-boiling compounds is important; but these do not duplicate the total flavor of most products (Ryder, 1966).

Gas chromatography is an excellent method for separating components of a mixture, but cannot be used to determine the contribution of the various components to the flavor or aroma, nor to identify compounds (Burr, 1964; Bayer, 1966). Other methods, such as mass spectroscopy, infrared spectroscopy, or nuclear magnetic resonance, must be used to identify the flavor volatiles after separation (Hornstein and Teranishi, 1967; Elberg, 1967; Scott, 1969). The relative contributions of the different fractions to the total aroma are determined by human judges who must sniff the gas fractions as they leave the chromatograph, and/or taste known and

unknown mixtures of trapped volatiles to determine whether any alterations may have occurred (Burr, 1964; Bayer, 1966; Ryder, 1966; Elberg, 1966; and Teranishi and Mon, 1968). Thus, only experts with years of experience are qualified to make such evaluations, and even then, there are apt to be errors due to fatigue when complex mixtures are tested.

The American Society for Testing and Materials published an excellent review in 1967 of the correlations between subjective and objective methods of flavor analysis. Boggs et al. (1964) were able to correlate an increase in hexanal (as an indication of rancidity) over potato granules during storage with the judges' evaluation of flavor deterioration. McCarthy et al. (1963) found correlation of judges' flavor profiles of bananas and their gas chromatographic patterns. Guadagni et al. (1966) showed similar correlation between the judges' evaluations of Delicious apple essence and its corresponding chromatographic pattern, but found that the smallest chromatographic peak represented the volatile fraction with the most intense odor. Rhodes (1958) and Kendall and Neilson (1964) were not as successful in finding correlation between GLC and subjective odor analyses. Kendall and Neilson demonstrated that GLC may not detect certain odorants in low concentrations where they may produce important changes in the odor characteristics, and that it may not give an indication of odor blends or how new odors are produced.

Spices: Oils and Oleoresins

Spice oils and oleoresins are used instead of whole spices to flavor most fresh-pack and processed pickles, because they allow more intense and uniform flavor and permit microbiological control. Since spice oils and oleoresins are added to cucumber brines prior to pure culture fermentation (Etchells et al., 1968a), the constituents of these should be known in order to evaluate changes in the flavor components which might occur during microbial fermentation.

Most spice oils and oleoresins have been fractionated by gas chromatography. Only dill, garlic, capsicum, mustard, caraway, and pimenta will be discussed, since these are the major spices used to flavor dill pickle products. Carvone and phellandrene are the major constituents of oil of dill (Anethum graveolens L.). Guenther (1950) reported that phellandrene is responsible for the typical odor and flavor of the fresh herb and dominates the total flavor when there is less than 35% carvone present (carvone being found in higher concentrations in the seed). Commercial oil of dill may be "cut" or standardized with limonene, a terpene of citrus origin, which may equal or exceed on a weight basis the weight of the dill oil (Sethi, Nigam, and Rao, 1965).

The odorous components of garlic (Allium sativum) are released by enzymatic action from the parent compound alliin (Jacobs,

1951a). Enzymes have also been found to be essential to the release of volatiles in other substrates (Weurman, 1961; Fleming et al., 1968). Alliin is converted to allicin with the release of pyruvate and ammonia by the enzyme alliinase (Jacobs, 1951a; Guenther, 1952). Allicin spontaneously decomposes to give methyl- and allyl-sulfides, disulfides, and trisulfides (Oaks et al., 1964).

In contrast to garlic and dill, in which the volatile oils are responsible for the typical odor and flavor, the non-volatiles of capsicum (Capsicum frutescens) are the source of its flavor and "heat" (Rogers, 1966). Capsicum is, therefore, sold as an oleo-resin, containing fixed oil, capsaicin, pigments, sugars, and resins, but no essential oil (Mathew et al., 1971). Capsaicin, a substituted benzylamine derivative, is the pungent principle of capsicum (Rogers, 1966; Mathew et al., 1971).

The extremely volatile isothiocyanates of mustard oils (Brassica nigra or B. juncea) are responsible for their odor and flavor (Guenther, 1949, 1952).

Oil of caraway (Carum carvi) contains 50 to 60% carvone, and is usually diluted with limonene, as is dill (Guenther, 1950).

Pimenta leaf oil (Pimenta officinalis) has the odor and flavor of allspice, eugenol being present in a concentration greater than 81% for high grade oil (Guenther, 1950).

METHODS AND MATERIALS

Preparation of Pickles by Pure Culture Fermentation

Cultures

Three strains of *Lactobacillus plantarum*, two of *Pediococcus cerevisiae*, and mixtures of both were used in these studies. *L. plantarum* FBB-12 and *P. cerevisiae* FBB-39 were obtained through the courtesy of Dr. R. N. Costilow of the Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan. *L. plantarum* Nos. 1 and 4 and *P. cerevisiae* No. 3 were purchased from Chris Hansen Laboratories, Milwaukee, Wisconsin. Cultures were maintained in APT agar stabs with added calcium carbonate, and were transferred once daily in APT broth at least three days prior to their inoculation into cucumber samples. Incubation for the active broth cultures was 30 C; maintenance subcultures were incubated 24 hr at 30 C, stored at 4 C, and subcultured at 30-day intervals.

Brines

Covering brines contained water, salt, spices, and food coloring. Food grade lactic acid was added to acidify the brines to approximately pH 5.0. Each brine contained a small amount of yellow no. 5 (0.008% w/w) and 6.7% (w/w) salt, to result in an approximate concentration of 2.5 to 3.0% (w/w) salt after equilibration with the cucumbers.

Two commercial spice formulations, containing only spice oils and oleoresins, and three formulations combining whole spices with spice oils were used. Various spice mixtures were added to standard salt brines in the following amounts:

1XKD--single strength kosher dill formula, containing oil of dill weed, oil of mustard, oleoresin-oil of garlic, oleoresin capsicum, and polysorbate 80--0.09% (v/v)

2XKD--double strength kosher dill formula--0.18% (v/v)

3XKD--triple strength kosher dill formula--0.27% (v/v)

2XGD-KD--double strength garlic and dill, single strength mustard and capsicum--0.18% (v/v)

1XDC--single strength dill chip formula, containing oil of dill weed, oleoresin-oil of garlic, oleoresin capsicum, caraway oil, pimenta leaf oil, and polysorbate 80--0.47% (v/v)

2XDC--double strength dill chip formula--0.94% (v/v)

WSG--3 g per quart of a mixed pickling spice containing pepper, capsicum, ginger, bay leaves, mustard, cassia, coriander, cloves, dill, celery, and fennel, plus 0.06% (v/v) oleoresin-oil of garlic

WSG2--3 g per quart of selected whole spices (ginger, cassia, cloves, and half of the bay leaves were removed from the whole mixed pickling spice), plus 0.06% (v/v) oleoresin oil of garlic

WSGD2--3 g per quart of selected whole spices, plus 0.06% (v/v) oleoresin-oil of garlic and 0.07% (v/v) oil of dill weed.

The spice oils were added to the covering brines during preparation. The whole spices were soaked in 70% ethanol overnight, dried in a vacuum oven, and added to the jars as they were inoculated, taking care that each jar received only one capsicum berry.

The cover brines were heated to 180 F to destroy asporogenous, vegetative mocroorganisms, and were then kept at 4 C until used the following day.

Cucumbers

Whole no. 2 cucumbers were used in all experiments. The cucumbers, jars, and caps were obtained through the courtesy of a Michigan pickle processor. The cucumbers were stored at 4 C on the day of receipt, and on the next day were washed and packed according to the patent by Etchells et al. (1968). Cucumbers were placed in a wire basket with a wire cover and were immersed in 180-185 F water for five minutes, bringing the surface temperature of the cucumbers to 140-160 F. The blanched cucumbers were drained and packed by hand into sterile quart jars, 560 to 570 g per jar. Plastic gloves dipped periodically into a solution containing 100 ppm chlorine were used in handling the cucumbers. The jars were sterilized separately with aluminum foil covers; the lids were heated at the time of use in 180 F water which had been acidified with food grade lactic acid to pH 3.0 or less. The jars containing the blanched cucumbers were immediately filled with 375 to 385 ml cold brine, capped, and labeled as to spice formulation and inoculum. After the cold brine and hot cucumbers equilibrated to a temperature

of approximately 86 F, an inoculum of 10^9 cells of the desired pure culture or mixture of pure cultures was aseptically added to each jar. The jars were tightly recapped and incubated at 86 F (30 C) for four days. After this primary fermentation, the product was stored at room temperature.

Six to ten jars per spice formulation were inoculated with each inoculum variable. One jar was used for each sampling period and extra jars were retained for organoleptic evaluation and chromatographic analyses. The same number of control jars were packed for each spice formulation. One control, designated C1, contained blanched cucumbers, but no inoculum. A second control, C2, also contained blanched cucumbers and was not inoculated, but was acidified with food grade (85%) lactic acid to a level of approximately 0.8% lactic acid. A third control was included in the earliest experiments: washed cucumbers, receiving no heat shock, uninoculated and unacidified. These were not included in later experiments because all produced a gaseous natural fermentation, but the pH was greater than 5.0.

Chemical Analyses of Pickles

The following chemical analyses were used to follow the fermentation: pH, titratable acidity, reducing sugar, total plate

count, and salt. The control jars and all samples were analyzed after 4, 8, and 30 days. One lot of pickles was also analyzed after 60 days storage.

pH

A Beckman model 1019 research pH meter was used to determine the pH of a portion of each brine sample.

Titratable Acidity

Ten milliliters of brine were titrated to a phenolphthalein endpoint with 0.1000 N sodium hydroxide. The results were calculated as per cent lactic acid.

Reducing Sugar

The sugar was extracted from each sample in the following manner. Equal weights of cucumber and brine were homogenized in a Waring blendor. To 20.0 g of blended sample were added 2.0 g of calcium carbonate and 200-300 ml distilled water. This mixture was boiled for thirty minutes, cooled, and then transferred to a 500-ml volumetric flask. Two to three milliliters saturated neutral lead acetate were added slowly, until the supernatant fluid was clear. Distilled water was added to bring the mixture to a volume of 500 ml; the solution was thoroughly mixed and filtered through

Whatman No. 2 paper. One gram anhydrous potassium oxalate was added to precipitate the excess lead; the product was mixed and refiltered.

One milliliter of the resulting filtrate was diluted with one ml distilled water and analyzed by the Folin-Wu micro method for determining reducing sugars (A.O.A.C., 1965). Concentrations of 0.1, 0.2, and 0.5 mg standard dextrose solutions were used as a basis for calculating the per cent reducing sugar in the samples.

The per cent reducing sugar in the sample was calculated by the following equation:

$$\text{per cent reducing sugar} = \frac{(c_s)(A_x)}{A_s} \times d \times \frac{500 \text{ ml}}{1000 \text{ mg/g}} \times \frac{100}{20 \text{ g}}$$

where:

c_s = concentration of dextrose standard

A_s = absorbance of dextrose standard at 420 nm

A_x = absorbance of sample at 420 nm

d = dilution factor.

The per cent conversion of reducing sugar to lactic acid was calculated as follows, using the data from cucumbers fermented by L. plantarum FBB-12 and P. cerevisiae FBB-39 (Table 3) as an example:

Example:

initial reducing sugar:

$$\frac{(\%\text{ sugar in unfermented control})\ (\text{total wt. cucumbers} + \text{brine in jar})}{100}$$

= g initial reducing sugar in jar

$$\frac{(1.27)\ (955\text{ g})}{100} = 12.1\text{ g reducing sugar initially}$$

final or residual sugar:

$$\frac{(0.49)\ (955\text{ g})}{100} = 4.7\text{ g residual reducing sugar}$$

reducing sugar utilized:

(12.1 g) - (4.7 g) = 7.4 g reducing sugar utilized

theoretical yield of lactic acid, assuming 100% conversion:

7.4 g lactic acid from 7.4 g sugar

actual yield of lactic acid:

0.72% acid in brine after fermentation
- 0.10% acid in original brine
0.62% acid produced by fermentation

$$\frac{(0.62)\ (955\text{ g})}{100} = 5.9\text{ g lactic acid produced}$$

$$\frac{5.9}{7.4} \times 100 = 80\%\text{ conversion of sugar to acid}$$

Total Count

Dilutions of sample brines were plated on All Purpose plus Tween Agar (APT; Difco Laboratories, Detroit), which contains the

following constituents per liter of distilled water: 7.5 g Bacto-yeast extract; 12.5 g Bacto-tryptone; 10.0 g Bacto-dextrose; 5.0 g sodium citrate; 0.001 g thiamine hydrochloride; 5.0 g sodium chloride; 5.0 g dipotassium phosphate; 0.14 g manganese chloride; 0.8 g magnesium sulfate; 0.04 g ferrous sulfate; 0.2 g sorbitan monooleate complex; 15 g Bacto-agar; and with final pH 6.7. Plates were incubated at 30 C and counted after 48 hrs. Colonies were observed for the typical morphology of L. plantarum and/or P. cerevisiae.

Salt

One milliliter of sample brine was diluted with four ml of distilled water and the per cent salt determined using Quantab Chloride Titrator No. 1176 (Ames Division of Miles Laboratories, Inc., Elkhart, Ind.).

Organoleptic Analyses of Pickles

All controls and samples, after complete fermentation, were subjected to an expert panel of a Michigan pickle processing firm who rated the samples on a five-point hedonic scale. Three of the most acceptable samples, flavored with the double strength (2X) dill chip spice formulation, and a commercial pickle sample (Kosher-style dill pickles) were submitted to two separate taste panels for flavor evaluation based on a nine-point hedonic scale.

The results were analyzed for preference by the analysis of variance procedure (Kramer and Twigg, 1966).

Chromatographic Analyses of Pickles

Column

The column used for the chromatographic analyses was pre-packed by Analabs, North Haven, Conn. The ten-foot stainless steel column was packed with 60 to 80 mesh, acid washed, silanized Chromosorb W which had been coated with 10% Carbowax 20M.

The column was conditioned for 72 hours at 210 C prior to injection of the samples.

Gas Chromatograph

A Hewlett-Packard model 5750 B research chromatograph, equipped with a Moseley 7128 A dual pen strip chart recorder (F&M Scientific Co., Avondale, Pa.) was used for the analyses. Both flame ionization and electron capture (EC) detectors were used. Energy in the form of beta-radiation was supplied to the electron capture detector by a tritium foil. A stream splitter was attached to the column exit to send one-half of the effluent to each of the detectors.

The operating parameters of the chromatograph were:

Column:	10' × $\frac{1}{8}$" stainless steel, prepacked (Analabs)
Support:	60 to 80 mesh Chromosorb W, acid washed, silanized
Coating:	10% Carbowax 20M
Sample Size:	0.5 ml for all samples and standards
Carrier Gas:	Helium at pressure of 40 psi
	50 ml per min flow rate through column
	25 ml per min flow rate through each detector
Purge Gas:	90% Argon-10% Methane, High Purity (Matheson Co., East Rutherford, New Jersey), at pressure of 30 psi
	63 ml per min through EC detector only
Hydrogen:	12 psi
Air:	33 psi
Temperatures:	Detectors--130 C
	Injection Port--110 C
	Column--80 C
Chart Speed:	1.0 in per min for first 5 min, then 0.25 in per min
Sensitivity and Attentuation:	10 × 1 for flame ionization
	10^2 × 4 for electron capture
Pulse Interval (EC):	50 μsec

Preparation of Samples and Standards

Half of the sample jar lids for the pickles made with a 2XDC spice formulation were fitted with a rubber serum bottle septum. The septa were inserted into a hole drilled in each of the lids and were sealed to the lids with epoxy cement.

Spice formulations for chromatographic analyses were prepared in the same fashion as the brines that were added to the cucumbers, but the cucumbers were omitted. Each component of the spice formulation in its proper concentration was added singly to an acidified salt brine of the same composition as that added to the pickle samples prior to fermentation. The brine-spice mixture (385 ml) was added to sterile jars, mixed, heated to 180 F, and cooled. The headspace of these single spices and of a brine containing the entire spice formulation was sampled through rubber septa in the jar lids, and the chromatographic profiles obtained were used as standards for comparison with the fermented samples.

Headspace gas samples were obtained with a Pressure-Lok series B gas syringe, fitted with a 23 gauge × 2 inch luer-lok needle (Precision Sampling Corporation, Baton Rouge, La.). The syringe was washed between samples by withdrawing hexane from five different baths, rinsing the barrel assembly several times at each bath, and drying in air between washings.

Interpretation of Results

No attempt was made to quantitate or to identify the chromatographic peaks. Interpretation of results was based on a visual examination for the presence or absence of peaks, the relative ratios of peak heights, and retention times.

RESULTS AND DISCUSSION

Chemical Analyses

Changes in Reducing Sugar, Titratable Acidity, and pH

All heated unacidified controls receiving no pure culture inoculum (C1) supported the growth of sporeforming bacteria (long rods, presumably clostridia with some bacilli near the headspace of the jars) which survived the heat shock treatment. The cucumbers became soft, and a pH greater than 5.0 was produced. These controls were not included in subsequent chemical or organoleptic analyses.

Thus, the heated, acidified, uninoculated controls (C2) were used as a reference for the amount of reducing sugar originally present in the cucumbers. It was assumed that the reducing sugar content of the control samples did not change, since these were not fermented. Slight variations in sugar were noted (Table 1), but these could be accounted for in part by the variable amount of reducing sugar in different cucumbers. The Folin-Wu procedure for determining reducing sugar was used because the principal sugars in

TABLE 1. -- pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar in heated, acidified, uninoculated control samples in various spice formulations.

Spice Formulation	pH	Titratable Acidity (%)	Reducing Sugar (%)
1XKD	3.21	0.66	1.03
2XKD	3.12	0.83	1.39
2XGD-KD	3.13	0.81	1.28
1XDC	3.10	0.82	1.41
2XDC	3.14	0.82	1.44
2XDC	3.11	0.83	1.14
2XDC	3.14	0.82	1.32
WSG	3.13	0.79	1.37
WSG2	3.12	0.79	1.18
WSGD2	3.18	0.79	1.18
Average	3.14	0.80	1.27

cucumbers are glucose and fructose, sucrose being present in only trace amounts (Jacobs, 1951b). The average reducing sugar content of all control samples (1.27%) was used as the initial level of sugar in the jars.

The amount of reducing sugar present in the fresh cucumbers was calculated by dividing the average total grams of sugar per control jar by 570 g, the weight of cucumbers in the jars (see page 28). This figure, 2.13%, agrees with the value of 2.17% reported by Jones and Etchells (1943, Table 1) for cucumbers of the size used in these experiments.

Data in Tables 2, 3, and 4 indicate that the different spice formulations had no significant effect on the fermentation produced by the pure cultures. Differences in data obtained when spice variables were repeated (c.f., 2XKD and 2XDC) were probably due to the use of different lots of cucumbers and to experimental errors in sampling and technique. Due to the bacteriostatic effects of certain spice oils (e.g., mustard and garlic), the organisms might be expected to grow better and produce a more rapid fermentation in the whole spiced brines. The data indicate only a slight advantage to the fermentation by the FBB-12 strain of L. plantarum (Tables 3 and 4). Also, as the concentration of the spice oil mixtures increased, there was some inhibition of fermentations by both L. plantarum and P. cerevisiae (Tables 2 to 4).

TABLE 2. --Changes in pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar during pure culture fermentation of cucumbers in various spice formulations by Pediococcus cerevisiae FBB-39.

Spice Formulation	pH			Titratable Acidity (as lactic) (%)			Reducing Sugar (%)		
	Days			Days			Days		
	4	8	30	4	8	30	4	8	30
1XKD	3.67	3.56	3.46	0.34	0.41	0.47	0.89	0.53	0.53
2XKD	3.59	3.58	3.49	0.43	0.43	0.46	--	0.50	0.47
2XKD	3.76	3.68	3.65	0.27	0.38	0.47	0.75	1.00	0.97
3XKD	3.56	3.68	3.78	0.30	0.34	0.24	0.52	0.61	0.68
2XGD-KD	3.81	3.77	3.61	0.28	0.28	0.38	0.94	0.79	1.00
1XDC	3.73	3.59	3.59	0.34	0.36	0.39	0.97	1.00	1.14
2XDC	3.81	3.67	3.64	0.30	0.34	0.36	0.95	0.92	1.08
2XDC	3.78	3.69	3.56	0.22	0.33	0.33	0.79	0.77	0.87
WSG	3.72	3.60	3.60	0.34	0.36	0.40	0.84	0.77	0.94
WSG2	3.69	3.69	3.50	0.28	0.35	0.40	0.77	0.59	0.77
Average	3.71	3.65	3.59	0.31	0.36	0.39	0.82	0.75	0.84

Note: Brines were acidified to an initial pH of 5.0, approximately 0.1% titratable acidity (calculated as lactic); all jars initially contained approximately 1.27% reducing sugar.

TABLE 3. --Changes in pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar during pure culture fermentation of cucumbers in various spice formulations by the combination L. plantarum FBB-12 and P. cerevisiae FBB-39.

Spice Formulation	pH			Titratable Acidity (as lactic) (%)			Reducing Sugar (%)		
	Days			Days			Days		
	4	8	30	4	8	30	4	8	30
1XKD	3.64	3.56	3.33	0.34	0.44	0.66	0.64	0.64	0.39
2XKD	3.58	3.43	3.42	0.48	0.52	0.50	--	0.24	0.50
2XKD	3.66	3.51	3.31	0.30	0.48	0.68	0.94	0.66	0.65
3XKD	3.60	3.54	3.48	0.31	0.40	0.47	0.62	0.49	0.50
2XGD-KD	3.84	3.63	3.37	0.27	0.37	0.64	0.97	0.84	0.78
1XDC	3.49	3.29	3.19	0.49	0.64	0.92	0.74	0.67	0.53
2XDC	3.69	3.46	3.26	0.38	0.50	0.73	1.05	0.79	0.67
2XDC	3.83	3.66	3.45	0.26	0.34	0.56	0.74	0.59	0.54
WSG	3.32	3.24	3.12	0.62	0.74	1.02	0.66	0.54	0.28
WSG2	3.40	3.69	3.15	0.57	0.64	0.97	0.51	0.51	0.03
Average	3.60	3.50	3.31	0.40	0.51	0.72	0.76	0.60	0.49

Note: Brines were acidified to an initial pH of 5.0, approximately 0.1% titratable acidity (calculated as lactic); all jars initially contained approximately 1.27% reducing sugar.

TABLE 4. -- Changes in pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar during pure culture fermentation of cucumbers in various spice formulations by Lactobacillus plantarum FBB-12.

Spice Formulation	pH			Titratable Acidity (as lactic) (%)			Reducing Sugar (%)		
	Days			Days			Days		
	4	8	30	4	8	30	4	8	30
1XKD	3.44	3.31	3.34	0.48	0.66	0.58	0.61	0.26	0.24
2XKD	3.59	3.39	3.22	0.34	0.61	0.83	--	0.48	0.18
2XKD	3.50	3.34	3.24	0.43	0.63	0.80	1.00	0.60	0.46
3XKD	3.40	3.23	3.35	0.44	0.68	0.64	0.36	0.36	0.34
2XGD-KD	3.91	3.32	3.25	0.22	0.70	0.90	0.86	0.50	0.35
1XDC	3.40	3.28	3.11	0.50	0.62	0.96	0.95	0.79	0.39
2XDC	3.50	3.33	3.23	0.42	0.51	0.78	0.76	0.92	0.53
2XDC	3.49	3.21	3.09	0.37	0.48	0.80	0.72	0.67	0.28
WSG	3.42	3.19	3.06	0.64	0.80	1.02	0.79	0.44	0.33
WSG2	3.29	3.44	3.09	0.60	0.71	0.98	0.38	0.12	0.10
Average	3.49	3.30	3.20	0.44	0.64	0.83	0.71	0.51	0.32

Note: Brines were acidified to an initial pH of 5.0, approximately 0.1% tritable acidity (calculated as lactic); all jars initially contained approximately 1.27% reducing sugar.

The data of Tables 2, 3, and 4 are also illustrated in Figures 1, 2, and 3 that show the average decreases in pH and reducing sugar and increases in titratable acidity for all spice formulations during pure culture fermentations by P. cerevisiae FBB-39, L. plantarum FBB-12, and the mixed cultures. The figures show that the fermentation is rapid during the first two days and is essentially complete after the first week. Lactobacilli are more acid-tolerant and generally continue to produce a small amount of acid, even after 30 days.

Tables 5 and 6 include data that indicate the differences in fermentations produced by various strains of P. cerevisiae, L. plantarum, and mixtures of both in a double strength dill chip formulation and a whole spice formulation. Figure 4 summarizes the effects of each of the inoculum variables on the fermentation in the double strength dill chip formulation, showing final titratable acidity and reducing sugar after 60 days. Pediococci produced the least acid and utilized less than half of the fermentable sugars. L. plantarum strain No. 1 produced the most acid, leaving only a trace of reducing sugar in the cucumbers. The combined pure cultures of L. plantarum strain No. 1 and P. cerevisiae strain No. 3 produced the same level of acid as the Lactobacillus alone. L. plantarum strains 12 and 4 are intermediate acid-producers.

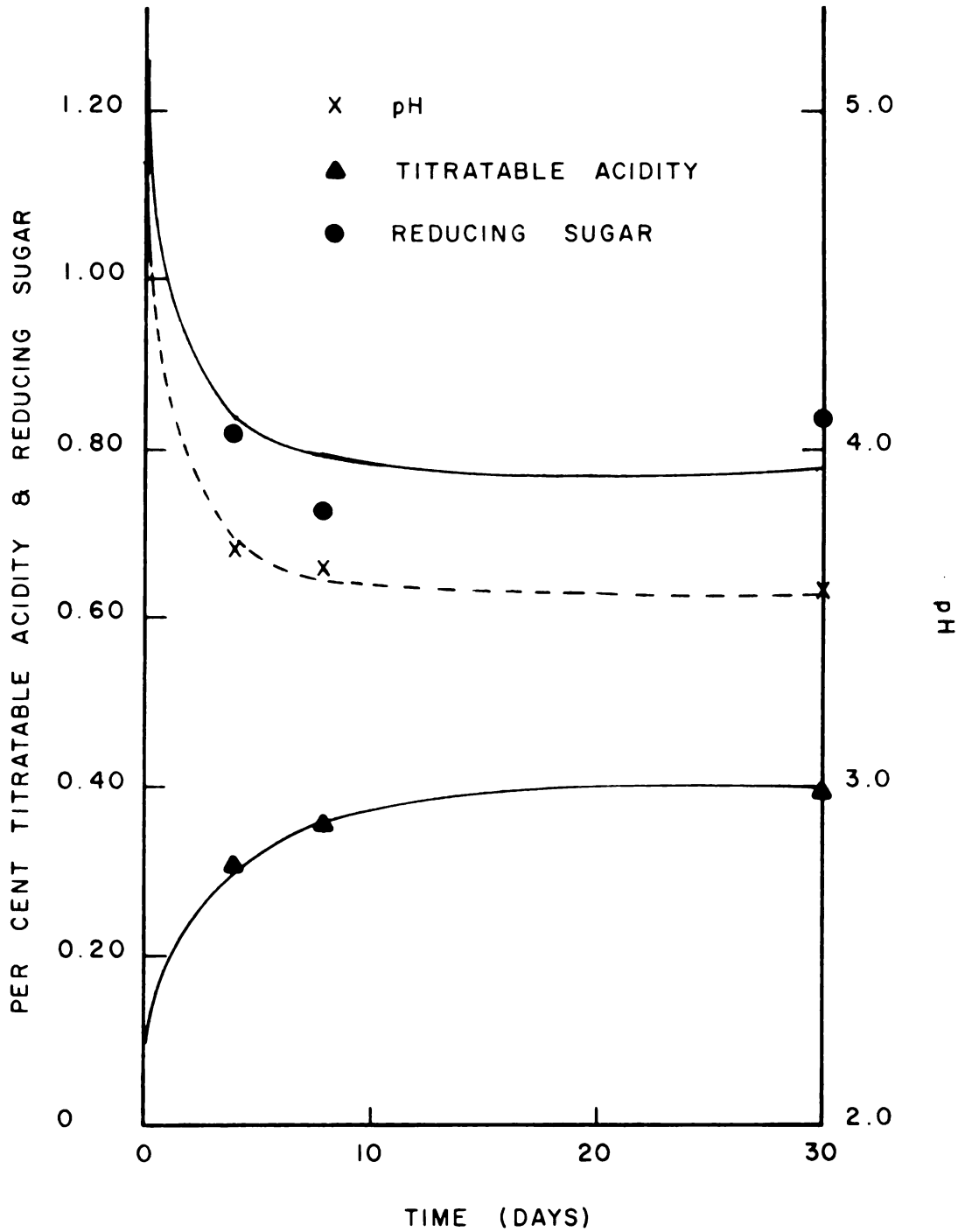


FIGURE 1. -- Changes in pH, per cent reducing sugar, and per cent titratable acidity (calculated as lactic acid) during pure culture fermentation of cucumbers by Pediococcus cerevisiae FBB-39.

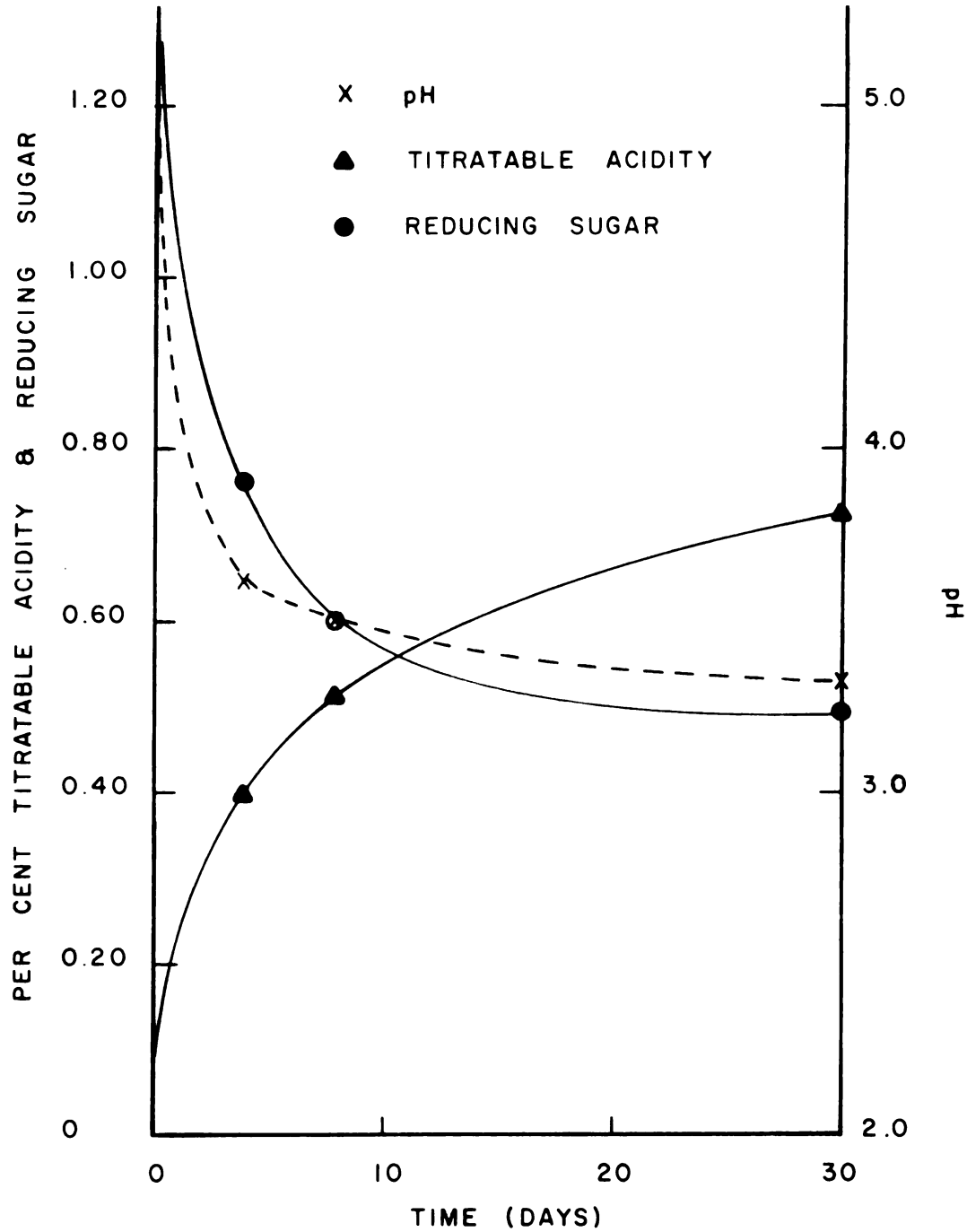


FIGURE 2. -- Changes in pH, per cent reducing sugar, and per cent titratable acidity (calculated as lactic acid) during pure culture fermentation of cucumbers by Lactobacillus plantarum FBB-12 and Pediococcus cerevisiae FBB-39.

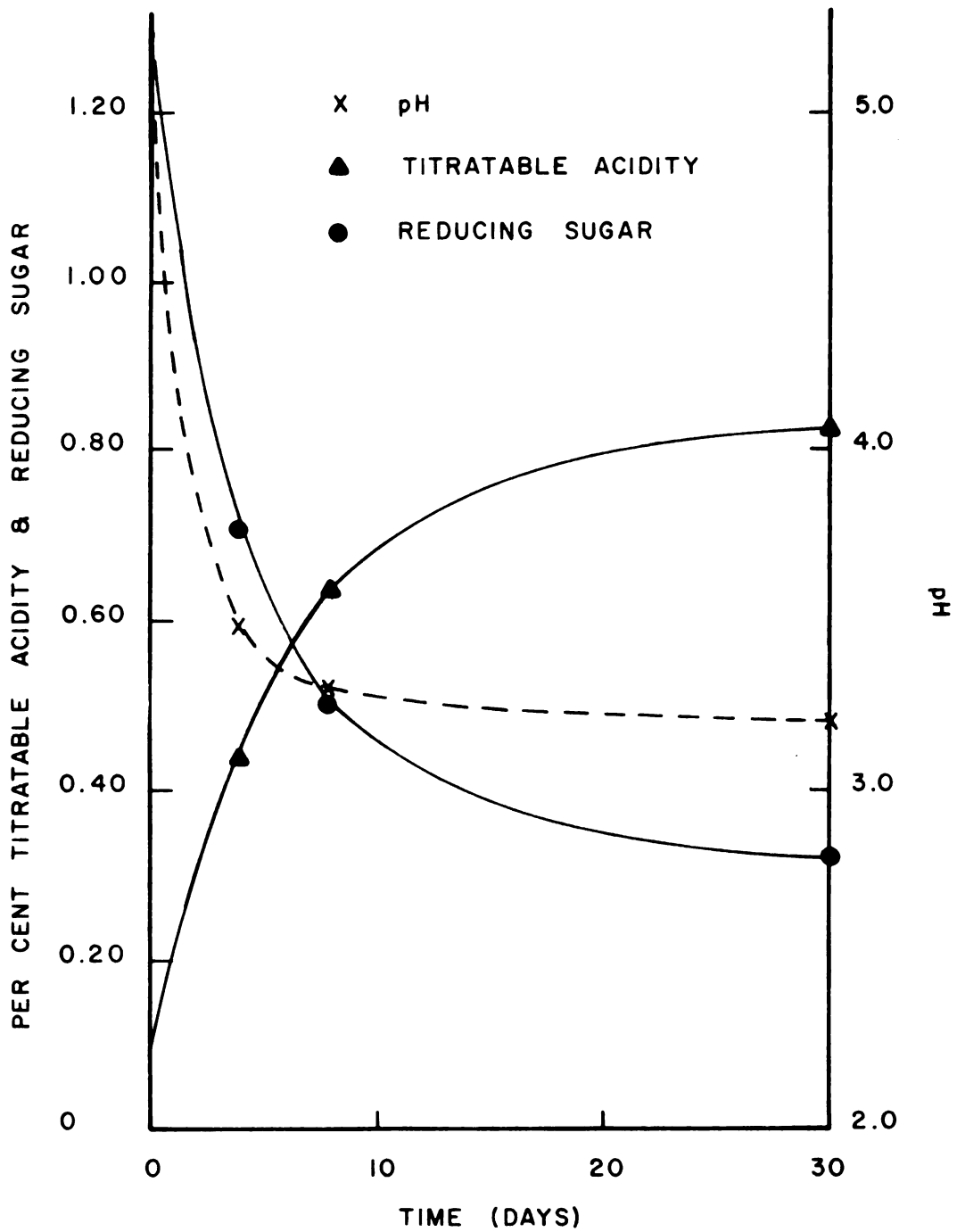


FIGURE 3. -- Changes in pH, per cent reducing sugar, and per cent titratable acidity (calculated as lactic acid) during pure culture fermentation of cucumbers by Lactobacillus plantarum FBB-12.

TABLE 5. -- Changes in pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar during pure culture fermentation of cucumbers in brine flavored with the double strength dill chip spice formulation by various strains of Lactobacillus plantarum and Pediococcus cerevisiae.

Strain of Fermenting Organism	pH				Titratable Acidity (as lactic) (%)				Reducing Sugar (%)			
	Days				Days				Days			
	4	8	30	60	4	8	30	60	4	8	30	60
Pc 39	3.78	3.69	3.56	3.70	0.22	0.33	0.33	0.33	0.79	0.77	0.87	0.77
Pc 3	3.70	3.61	3.54	3.53	0.36	0.37	0.44	0.47	0.82	0.80	0.88	0.79
Lp 12 + Pc 39	3.83	3.66	3.45	3.25	0.26	0.34	0.56	0.71	0.74	0.59	0.54	0.38
Lp 1 + Pc 3	3.45	3.33	3.06	3.10	0.56	0.66	1.14	1.24	0.54	0.42	0.02	0.05
Lp 4 + Pc 3	3.71	3.54	3.28	3.28	0.38	0.45	0.76	0.72	0.69	0.75	0.38	0.46
Lp 12	3.49	3.21	3.09	3.20	0.37	0.48	0.80	0.93	0.72	0.67	0.28	0.23
Lp 1	3.36	3.25	3.03	3.08	0.61	0.85	1.20	1.22	0.51	0.38	0.02	0.03
Lp 4	3.57	3.33	3.18	3.28	0.60	0.78	0.88	0.84	0.69	0.58	0.35	0.41

Note: Brines were acidified to an initial pH of 5.0, approximately 0.1% titratable acidity (calculated as lactic); all jars initially contained approximately 1.27% reducing sugar.

Lp = Lactobacillus plantarum; Pc = Pediococcus cerevisiae

TABLE 6. -- Changes in pH, per cent titratable acidity (calculated as lactic acid), and per cent reducing sugar during pure culture fermentation of cucumbers in a brine flavored with selected whole spices plus garlic and dill by various strains of Lactobacillus plantarum and Pediococcus cerevisiae.

Strain of Fermenting Organism	pH				Titratable Acidity (as lactic) (%)				Reducing Sugar (%)			
	Days				Days				Days			
	4	8	30	60	4	8	30	60	4	8	30	60
Pc 3	3.78	3.67	3.16	3.20	0.30	0.36	0.89	0.93	0.64	0.73	0.25	0.23
Lp 1 + Pc 3	3.33	3.25	3.04	3.02	0.72	0.84	1.24	1.20	0.46	0.38	0.02	0.05
Lp 4 + Pc 3	3.62	3.44	3.24	3.22	0.44	0.59	0.74	0.78	0.69	0.65	0.38	0.36
Lp 1	3.34	3.25	2.98	3.12	0.68	0.84	1.14	1.19	0.38	0.28	0.08	0.05
Lp 4	3.43	3.31	3.14	3.22	0.59	0.74	0.80	0.80	0.54	0.45	0.30	0.36

Note: Brines were acidified to an initial pH of 5.0, approximately 0.1% titratable acidity (calculated as lactic); all jars initially contained approximately 1.27% reducing sugar.

Lp = Lactobacillus plantarum; Pc = Pediococcus cerevisiae

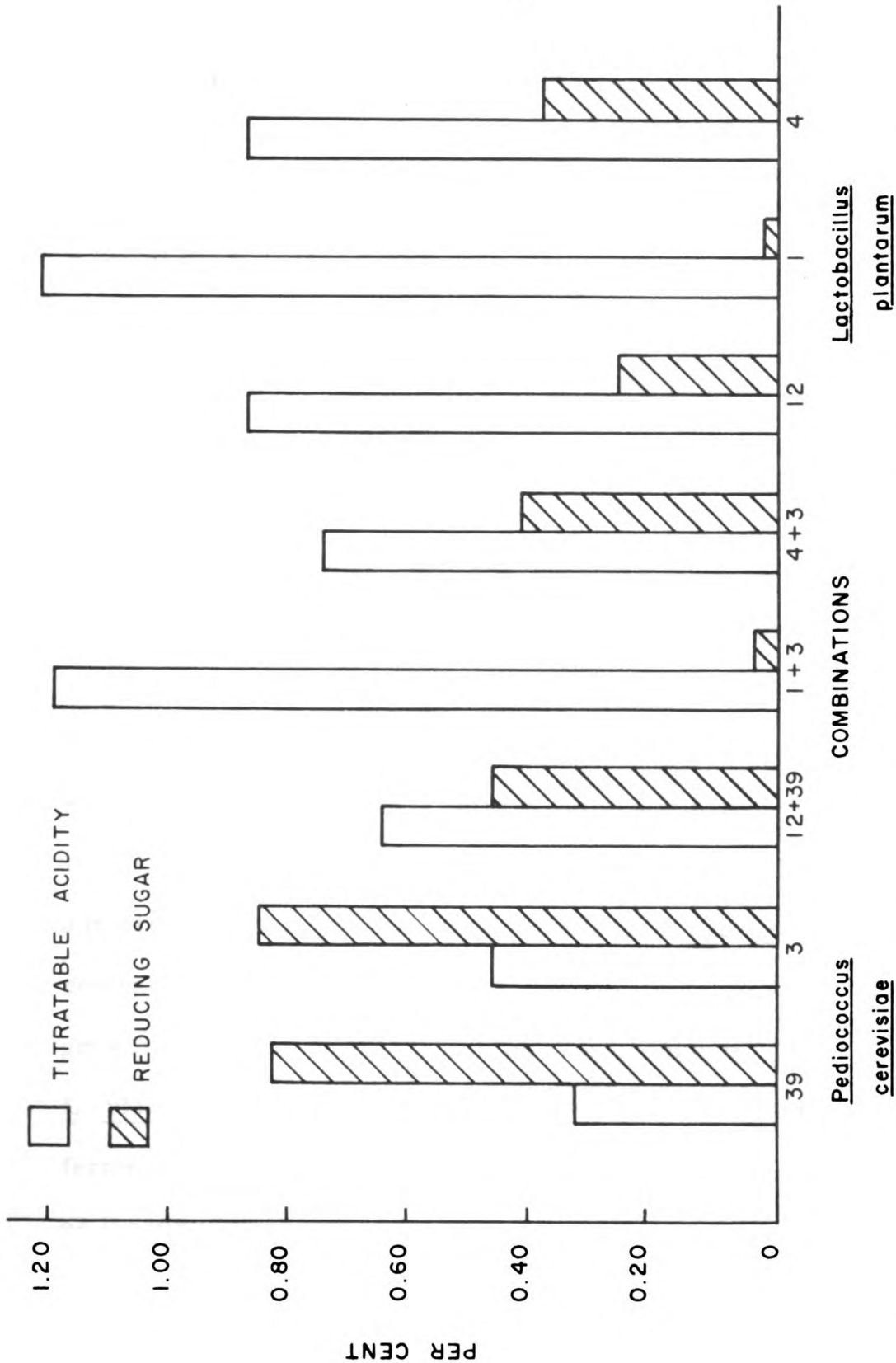


FIGURE 4. -- Final per cent reducing sugar and titratable acidity (calculated as lactic acid) after 60 days in pickles fermented by various strains of Lactobacillus plantarum and Pediococcus cerevisiae.

The combination of these strains with strains of P. cerevisiae slightly depressed the amount of acid produced.

The final pH or titratable acidity may be used to evaluate acid fermentations. Different strains of Lactobacillus will produce varying amounts of acid, but there is not necessarily a correlation between the amount of acid produced and the hydrogen ion concentration (Pederson and Bagg, 1944). The final pH depends on the buffering capacity of the medium and on the relative proportion of lactic and acetic acids. (Homofermentative lactobacilli may produce 1 - 10% acetic acid, in addition to lactic acid.) The cucumber-brine system is poorly buffered; thus the hydrogen ion concentration increases rapidly, inhibiting further growth of the organisms. However, Pederson and Bagg (1944) indicated that neither the pH nor titratable acidity alone were limiting for fermentation. These authors contended that the combined effects of hydrogen ion concentration, undissociated lactic and acetic acids, and/or other growth products result in inhibition of fermentation. Since L. plantarum strain No. 1 is capable of utilizing essentially all the fermentable sugars in cucumbers, the level of carbohydrate could be limiting in this case.

Data showing the per cent conversion of sugar to lactic acid are presented in Table 7. L. plantarum strain No. 1 appeared

TABLE 7. -- Per cent conversion of sugar to lactic acid during pure culture fermentation of cucumbers by various strains of Lactobacillus plantarum and Pediococcus cerevisiae.

Strain of Fermenting Organism	Spice Formulation	Reference Table	g Sugar Utilized/Jar	g Lactic Acid Produced/Jar	% Conversion
Pc 39	avg of all	T2	4.1	2.8	68
Lp 12	avg of all	T4	9.0	7.0	78
Lp 12 + Pc 39	avg of all	T3	7.4	5.9	80
Pc 39	2XDC	T5	4.7	2.2	47
Lp 12	2XDC	T5	9.9	7.9	80
Lp 12 + Pc 39	2XDC	T5	8.5	5.8	68
Pc 3	2XDC	T5	4.6	3.5	76
Lp 1	2XDC	T5	11.8	10.7	91
Lp 4	2XDC	T5	8.2	7.1	87
Lp 1 + Pc 3	2XDC	T5	11.6	10.9	94
Lp 4 + Pc 3	2XDC	T5	7.7	5.9	77
Pc 3	WSGD2	T6	9.9	7.9	80
Lp 1	WSGD2	T6	11.6	10.4	90
Lp 4	WSGD2	T6	8.7	6.7	77
Lp 1 + Pc 3	WSGD2	T6	11.6	10.5	90
Lp 4 + Pc 3	WSGD2	T6	8.7	6.5	75

Note: Lp = Lactobacillus plantarum; Pc = Pediococcus cerevisiae

to be the most efficient and P. cerevisiae FBB-39 the least efficient in this respect. Differences in spice formulations had no significant effect on the per cent conversion. No explanation can be given for the low value obtained for the conversion of sugar to lactic acid during the fermentation of cucumbers in a 2XDC brine by P. cerevisiae FBB-39. Mixtures of pure cultures of various strains of L. plantarum and P. cerevisiae had little effect on the final conversion produced during these fermentations. The fact that yields lower than the theoretical 100% conversion were obtained indicates that some of the sugar utilized by the bacteria was converted to other intermediary metabolites, building materials, and energy.

The Folin-Wu determination for reducing sugars was not too precise. The standard deviation for values obtained for any given inoculum variable was much too high to be accounted for by differences in cucumbers alone (see Tables 2 to 6). This method was chosen to obtain rapid approximations of the reducing sugar in cucumbers during various stages of fermentation by different pure cultures. Only a value for residual sugar after fermentation would be of interest to the pickle processor. Other methods for determining reducing sugar should be investigated.

Equilibration of salt at 2.5 to 3.0% (w/w) was complete in all jars by the fourth day.

Plate Counts

Total aerobic plate counts for all the inoculum and spicing variables are presented in Tables 8 to 12 (Appendix). There was no apparent effect of spice formulation on growth of the microorganisms. Initial (0-day) plate counts were made by adding 1 ml of the inoculum to 385 ml brine and plating subsequent serial dilutions. An initial count was not made for the 3XKD inoculum variables; the inoculum was assumed to be of the order of 10^6 organisms per ml, since these were of the same lot as the 1XKD and 2XKD samples. Similarly, the inoculum for 2XGD-KD samples was assumed to be approximately 10^9 organisms per ml. Initial counts were not determined for lots 3 and 4, due to a shortage of brine. Again, the inoculum was approximately 10^9 organisms per ml.

Plate counts were made for lot 1 spice formulations 1XKD, 2XKD, and 3XKD two days after inoculation. These counts showed increases to about 1×10^9 microorganisms/ml of brine for all the pure culture variables. All populations were declining by the fourth day. This corresponds to the rapid production of acid shown in Figures 1 to 3. The numbers of microorganisms continued to drop after the fourth day by as much as five log cycles by the end of 60 days (see Tables 10 and 11).

Organoleptic Evaluations

Samples of pickles fermented by each inoculum variable for each spice formulation were evaluated for flavor and acceptability by members of a Michigan pickle processing firm. The samples were rated on a five-point hedonic scale as excellent (5), good, fair, poor, or not acceptable (1). The unfermented, acidified controls for all the spice formulations were rated "poor" or "not acceptable." The control samples correspond to a commercial fresh-pack (unfermented) pickle, but were acidified with lactic rather than acetic acid. All of the pure culture fermented pickles had good texture and were crisp. The 1X and 2XKD and 2XGD-KD samples were generally rated "fair" with a slight bland flavor. The combination of P. cerevisiae FBB-39 and L. plantarum FBB-12 in the 2XKD flavored product was rated "good" by one of the judges, but "not acceptable" by another. Such differences in preference were noted for all spice formulations. The 3XKD samples were all unacceptable due to the high level of capsicum.

Products flavored by the whole mixed pickling spice plus garlic were too aromatic. Thus, only selected whole spices were used in subsequent whole spice formulations. Addition of oil of dill was necessary because the dill seeds added little flavor unless they were crushed. The WSG2 and WSGD2 formulations resulted in

products with very pronounced flavor and a notable "bite" of capsicum. These products were rated higher by some of the judges than others, depending on the intensity of flavor that was acceptable to them.

The dill chip formulation appeared to give the best flavored product. The flavor was more intense in the 2XDC samples; the 1XDC samples were slightly bland.

The acid fermentation had a decided effect on the flavor of the pickles, since all of the fermented samples could be distinguished from the acidified controls. The fermentations by different strains of L. plantarum and P. cerevisiae also had varying effects on the flavor of the final product. In a given spice formulation, strains of P. cerevisiae produced a more bland flavor. This could have been due to alteration of one of the spices and/or to the lower level of acid produced by these organisms. The fermentation by L. plantarum strain No. 1 singly and in mixed culture resulted in too high a level of acid to be acceptable. The consensus of the expert panel was that the double strength dill chip (2XDC) formulation in combination with the fermentations produced by either P. cerevisiae strain No. 3 or 39 or L. plantarum strain No. 4, or by mixed cultures of these, yielded the most acceptable products.

Samples of these pickles (2XDC - Pc 3, 2XDC - Lp 4, and 2XDC - Pc 3 + Lp 4) were submitted to taste panels for flavor

evaluation based on a nine-point hedonic scale (9 = liked extremely; 1 = disliked extremely). The judges individually rated each of the pure culture fermented samples and a commercial kosher-style dill pickle.

Statistical analysis of the results of two taste panels indicated no significant difference in preference between any of the pure culture fermented samples or between the pure culture fermented samples and the commercial pickle. The F values, calculated for sample variation (from raw data in Tables 15 and 16--Appendix), are presented in Tables 13 and 14; these were smaller in both cases than the value required for significance. Results of the first taste panel, Table 13, showed that there was a significant difference (P = 0.05) between judges. These results confirm the differences in preference, depending on the individual, that were noted during evaluation of the samples by the panel of experts.

Gas Chromatographic Analyses

The fermentation of cucumbers by *P. cerevisiae* resulted in notable changes in the flavor of the pickles, regardless of spice formulation, that were different from the flavors produced by *L. plantarum* and the *L. plantarum*-*P. cerevisiae* mixed cultures. These changes might be expected to be the result of interaction of

TABLE 13. -- Analysis of Variance showing significance of differences between a commercial product and pure culture samples fermented by Lactobacillus plantarum 4, Pediococcus cerevisiae 3, and the combined cultures, based on data from the first hedonic flavor evaluation.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Variance	Variance Ratio (F)	Tabular F	
					5%	1%
Total	75	215.41				
Samples	3	11.41	3.80	1.62	2.78	4.17
Judges	18	77.16	4.29	1.83	1.80	2.29
Residual	54	126.84	2.35			

Note: Raw data presented in Table 15 (Appendix).

TABLE 14. -- Analysis of Variance showing significance of differences between a commercial product and pure culture samples fermented by Lactobacillus plantarum 4, Pediococcus cerevisiae 3, and the combined cultures, based on data from the second hedonic flavor evaluation.

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Variance	Variance Ratio (F)	Tabular F	
					5%	1%
Total	79	201.99				
Samples	3	2.24	0.75	0.30	2.77	4.15
Judges	19	58.24	3.06	1.23	1.78	2.26
Residual	57	141.51	2.48			

Note: Raw data presented in Table 16 (Appendix).

the microorganisms themselves or their metabolic end-products with one or more of the spices. Because of the involvement of sulfur compounds in many biochemical reactions, the changes in flavor could be due to alteration of the volatile sulfur constituents of garlic. Brenner et al. (1955) found that beer yeasts produce many undesirable sulfur compounds in addition to alcohol. These authors showed the interrelationship of the sulfur compounds and how they could be altered by the yeasts' metabolism. Similarly, the metabolism of lactic acid bacteria might be expected to involve transformations of sulfur compounds.

Oaks et al. (1964) used dual channel gas chromatography to separate and identify the volatile sulfur constituents of garlic. Dual channel GC refers to the use of a single column and two different detectors. These authors used a flame ionization detector, because it is sensitive to a wide range of organic compounds, but not to inorganic gases or water vapor; and an electron capture detector, which is sensitive to only certain groups of organic compounds. The sensitivity of the electron capture detector varies with the electron affinities of such groups as conjugated carbonyls, halogens, and nitro- and sulfur-containing compounds. However, the detector response by electron capture is affected by oxygen and water vapor (Buttery and Teranishi, 1961). Oaks et al. (1964) found

electron capture to be more sensitive than flame ionization to the volatile sulfides in garlic, particularly the di- and trisulfides.

Based on the work of Oaks et al. (1964), dual channel gas chromatography was used in this experiment to determine whether the differences in the flavor of pickles fermented by pediococci and lactobacilli could be detected objectively. Carbowax 20M (polyethylene glycol) was used as a stationary phase because of its interactions with polar compounds. The Chromosorb W support was acid-washed to remove trace elements and treated with dimethyldichlorosilane (DMCS, Analabs) to make the surface more inert and thus minimize the adsorption of volatiles by the support surfaces. Certain restrictions were imposed upon the operating parameters by the use of the dual detectors and the choice of stationary phase. Loads of greater than 5% Carbowax 20M cause substrate "bleed" which affects the sensitivity of the electron capture detector. Substrate bleed can be minimized by using a column temperature less than 100 C. Maximum separation of components was obtained by using a column temperature of 80 C. The recommended carrier gas flow for the Hewlett-Packard 5750 B flame detector is 25 ml per min for $\frac{1}{8}$-inch columns. A purge gas (90% argon-10% methane) was required for the EC detector, to prevent overloading the detector cell with sample. The recommended ratio of purge gas to column

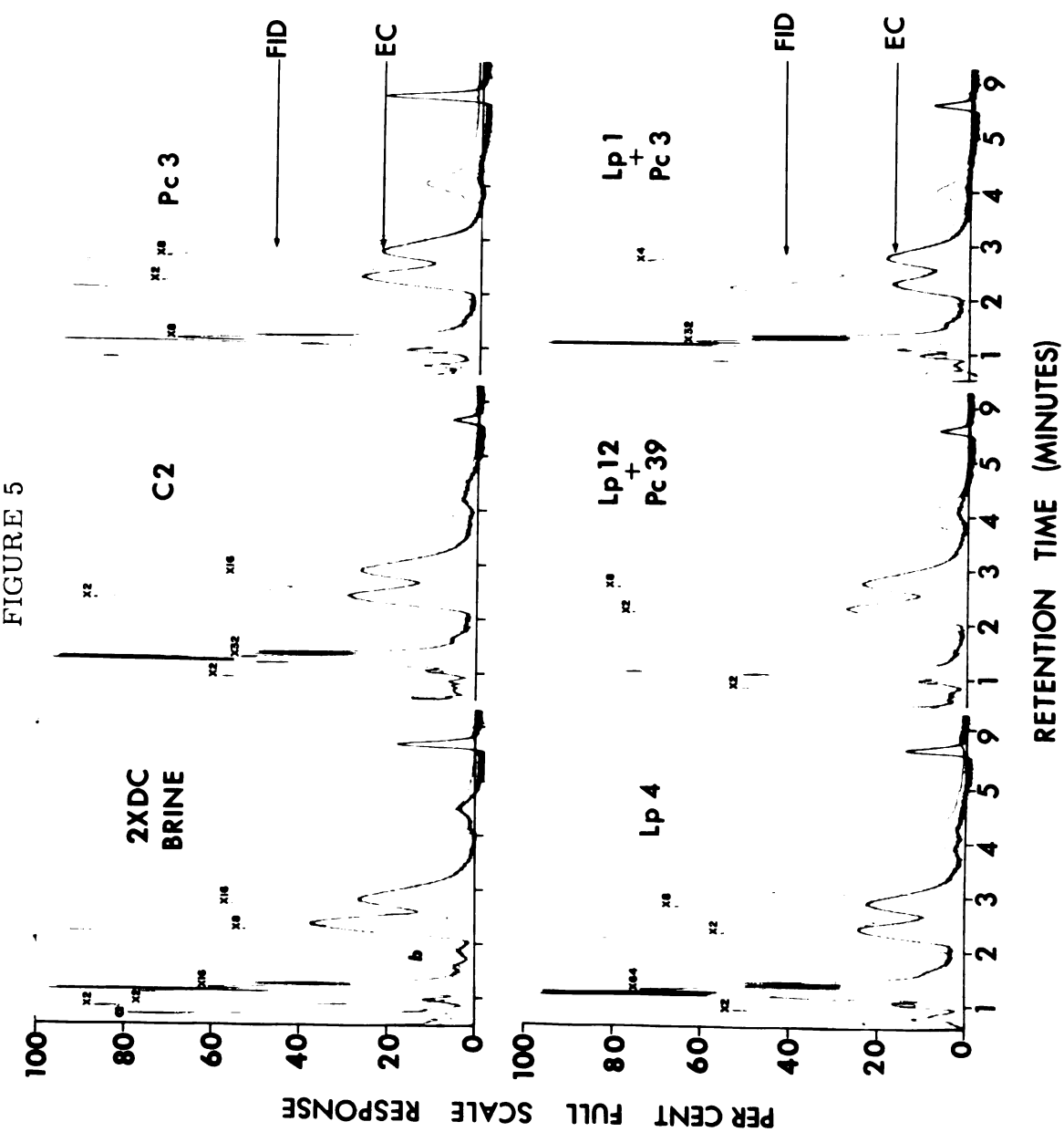
effluent was between 2.5 and 3 to 1. Moisture traps were connected to the gas lines to remove water vapor from the carrier and purge gases.

Representative chromatograms obtained upon GLC head-space analysis of 2XDC brine (no cucumbers), an acidified control, and samples fermented by strains of P. cerevisiae and L. plantarum singly and in mixed cultures are shown in Figure 5. No significant differences between the chromatograms for any of the pure culture variables or control samples were noted. No peaks were deleted and none were added as a result of the different fermentations. Slight differences in peak heights occurred but followed no pattern. These differences were probably due to lack of complete control over sample size and/or carry-over of volatiles adsorbed on the column from sample to sample. The column was allowed to condition for at least an hour between sample injections and overnight to minimize error due to residual sample.

Although there was no difference among chromatograms of the fermented samples or between the fermented samples and controls, it is interesting to note that the addition of cucumbers to the brine resulted in the disappearance of peaks a and b in Figure 5 (2XDC brine). No attempt was made to identify these peaks, except to say that peak a had the same retention time as a small peak

FIGURE 5. -- Gas chromatograms of the headspace vapors of a brine flavored with a double strength dill chip formulation, an unfermented spiced control, and pure culture fermented spiced samples analyzed by flame ionization (FID) and electron capture (EC).

FIGURE 5


2XDC
BRINE
C2
Pc 3
FID
EC
Lp 4
Lp 12
+
Pc 39
Lp 1
+
Pc 3
FID
EC
PER CENT FULL SCALE RESPONSE
RETENTION TIME (MINUTES)

present in chromatograms of the headspace vapors of both the garlic-brine and dill-brine standards. A larger peak with the same retention time as peak b was present in the chromatogram of the dill-brine standard.

The fact that both garlic and dill standards would contain components with the same retention time, but in different amounts, illustrates the possibilities for masking of small peaks by larger ones which may or may not be identical constituents (Elberg, 1967). With the maximum sensitivity of this instrument and the resolving power of the column used, it was not possible to characterize the differences between the fermentations by the various microorganisms.

Perhaps the flavor differences were due to changes in the non-volatile components, or to higher boiling volatile constituents, or to trace levels of volatiles which were not detected. Further work could be done using different stationary phases, such as Apiezon C, Apiezon L, or SF 96-50 (methyl silicone oil), which have been used for GLC analyses of other foods and flavorings. Pederson et al. (1964) showed that changes occurred in the lipid fraction of dill pickles during natural fermentation and suggested that these changes might affect the flavor of the product. Chromatographic analyses of the lipid fractions of pure culture fermented pickles might help to characterize the flavor differences. Other

suggestions for further investigation include using a larger sample size or concentrating the sample by increasing the vapor pressure (by raising the temperature) or by the addition of sodium sulfate (Bassette et al., 1962; Kepner, 1964). Concentrating the headspace vapor would alter the ratio of the various volatile constituents so that it would not give an accurate picture of their relative importance to the flavor, but might facilitate detection of differences between the fermented samples (Nawar and Fagerson, 1962).

SUMMARY AND CONCLUSIONS

The purpose of this study was to develop an adequate spice formulation for pure culture fermented pickles. The spice formulation was evaluated subjectively by expert and non-expert taste panels and objectively by GLC headspace analysis.

Several spicing and inoculum variables were studied. The different spice formulations had little effect on the fermentation by various pure cultures, but the organisms produced flavor differences in the finished pickles when the same spice formulation was used. Strains of L. plantarum produced the highest levels of lactic acid, and this acid affected the flavor of the final product. The L. plantarum strain No. 1 produced a pickle which was much too sour. Strains of P. cerevisiae produced a bland product, regardless of which spice formulation was used.

Samples of headspace vapor in jars of pickles fermented by strains of P. cerevisiae and L. plantarum, either singly or in mixed cultures, were analyzed by dual channel gas chromatography in an attempt to characterize the flavor differences. Under the conditions of the experiment, no differences between the headspace

vapors of any of the samples were evident. Therefore, the differences in flavor must have been due to changes in highly flavored volatile constituents present in trace amounts or to changes in the non-volatile flavor constituents.

A spice formulation containing oil of dill weed, oleoresin-oil of garlic, oleoresin capsicum, caraway oil, pimenta leaf oil, and polysorbate 80 was found to produce adequately flavored pure culture pickles when added in a concentration of 0.94% (v/v) to the covering brines. Samples of pickles processed in brine containing this spice formulation and fermented by P. cerevisiae strain No. 3, L. plantarum strain No. 4, and the mixture of both cultures were evaluated for flavor and acceptability by taste panels. The results indicated no overall significant difference in preference between these samples and a commercial kosher-style dill pickle. The product fermented by P. cerevisiae had a milder flavor, whereas the L. plantarum fermented product was more sour. The product fermented by the mixed culture was preferred by representatives of a Michigan pickle processing firm since the two cultures seemed to complement the effects of each other on the flavor. L. plantarum strain No. 4 accentuated certain flavor characteristics, while P. cerevisiae strain No. 3 attenuated other flavor characteristics, producing a desirable blend of flavors. However, preference in pickles varies so widely among individuals that these preliminary

evaluations indicate any of the three pure cultures (P. cerevisiae strain No. 3, L. plantarum strain No. 4, or the mixture of both) could produce a pickle of commercial potential. The pickle industry should test these products on a more widespread scale to determine whether the potential market justifies the cost of educating consumers to a new product.

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APPENDIX

TABLE 8. -- Changes in total plate counts (organisms/ml) of brines flavored by various spice formulations during pure culture fermentation by Pediococcus cerevisiae FBB-39.

Spice Formulation	Days			
	0	4	8	30
1XKD[a]	5.5×10^6	2.0×10^8	1.5×10^8	$> 3.0 \times 10^5$
2XKD[a]	3.7×10^6	2.2×10^7	5.7×10^7	$> 3.0 \times 10^5$
2XKD[b]	2.5×10^9	1.1×10^8	6.0×10^7	4.0×10^7
3XKD[a]	NO DATA	1.1×10^7	5.6×10^6	$> 3.0 \times 10^5$
2XGD-KD[b]	NO DATA	8.8×10^7	1.2×10^7	2.4×10^6
1XDC[c]	NO DATA	3.9×10^8	1.1×10^8	2.4×10^6
2XDC[c]	NO DATA	5.6×10^8	7.2×10^7	1.2×10^7
2XDC[d]	NO DATA	2.0×10^8	1.5×10^8	1.1×10^7
WSG[c]	NO DATA	7.0×10^8	1.3×10^8	8.4×10^7
WSG2[d]	NO DATA	2.2×10^8	3.1×10^8	6.0×10^7

[a]Lot 1

[b]Lot 2

[c]Lot 3

[d]Lot 4

TABLE 9. -- Changes in total plate counts (organisms/ml) of brines flavored by various spice formulations during pure culture fermentation by Lactobacillus plantarum FBB-12.

Spice Formulation	Days			
	0	4	8	30
1XKD[a]	9.5×10^5	2.1×10^7	1.9×10^{7e}	$> 3.0 \times 10^5$
2XKD[a]	NO DATA	8.5×10^6	1.8×10^6	2.7×10^3
2XKD[b]	7.1×10^8	1.2×10^8	6.9×10^7	1.6×10^6
3XKD[a]	NO DATA	9.5×10^5	8.0×10^4	2.1×10^4
2XGD-KD[b]	NO DATA	5.8×10^7	1.1×10^8	6.2×10^6
1XDC[c]	NO DATA	6.4×10^8	1.7×10^8	9.8×10^7
2XDC[c]	NO DATA	3.9×10^8	7.2×10^8	5.6×10^6
2XDC[d]	NO DATA	9.8×10^7	5.6×10^7	1.1×10^6
WSG[c]	NO DATA	8.4×10^8	5.2×10^8	8.1×10^7
WSG2[d]	NO DATA	9.0×10^8	6.0×10^8	7.4×10^7

[a]Lot 1

[b]Lot 2

[c]Lot 3

[d]Lot 4

[e]Estimated

TABLE 10. -- Changes in total plate counts (organisms/ml) of brines flavored by various spice formulations during pure culture fermentation by Pediococcus cerevisiae FBB-39 and Lactobacillus plantarum FBB-12.

Spice Formulation	Days			
	0	4	8	30
1XKD[a]	5.9×10^6	1.9×10^8	5.8×10^7	$> 3.0 \times 10^5$
2XKD[a]	2.4×10^6	2.4×10^7	1.1×10^7	$> 3.0 \times 10^5$
2XKD[b]	3.1×10^9	1.6×10^8	4.3×10^7	1.4×10^6
3XKD[a]	NO DATA	8.7×10^6	1.7×10^6	5.7×10^4
2XGD-KD[b]	NO DATA	7.1×10^7	2.3×10^7	1.9×10^6
1XDC[c]	NO DATA	2.6×10^8	1.3×10^8	5.3×10^7
2XDC[c]	NO DATA	5.1×10^8	1.2×10^8	6.9×10^7
2XDC[d]	NO DATA	2.2×10^8	8.2×10^7	1.2×10^6
WSG[c]	NO DATA	7.0×10^8	4.5×10^8	3.0×10^6
WSG2[d]	NO DATA	5.2×10^8	3.2×10^8	5.9×10^7

[a] Lot 1

[b] Lot 2

[c] Lot 3

[d] Lot 4

TABLE 11. -- Changes in total plate counts (organisms/ml) of brines flavored by the double strength dill chip formulation during pure culture fermentation by various strains of Pediococcus cerevisiae and Lactobacillus plantarum.

Strain of Fermenting Organism[a]	Days				
	0	4	8	30	60
Pc 39[b]	NO DATA	2.0×10^8	1.5×10^8	1.1×10^7	3.4×10^5
Pc 3[c]	9.7×10^8	1.7×10^8	1.6×10^8	5.2×10^7	1.5×10^7
Lp 12 + Pc 39[b]	NO DATA	2.2×10^8	8.2×10^7	1.2×10^6	$< 3.0 \times 10^4$
Lp 1 + Pc 3[c]	1.9×10^9	6.3×10^8	1.7×10^8	2.9×10^7	7.6×10^5
Lp 4 + Pc 3[c]	1.6×10^9	3.9×10^8	3.9×10^8	3.1×10^7	5.8×10^5
Lp 12[b]	NO DATA	9.8×10^7	5.6×10^7	1.1×10^6	$< 3.0 \times 10^4$
Lp 1[c]	9.5×10^8	6.4×10^8	7.5×10^8	6.8×10^7	1.2×10^6
Lp 4[c]	6.6×10^8	1.1×10^8	6.0×10^8	6.2×10^5	9.6×10^4

[a] Lp = Lactobacillus plantarum; Pc = Pediococcus cerevisiae

[b] Lot 4

[c] Lot 5

TABLE 12. -- Changes in total plate counts (organisms/ml) of brines flavored by selected whole spices plus garlic and dill during pure culture fermentation by various strains of Pediococcus cerevisiae and Lactobacillus plantarum.

Strain of Fermenting Organism[a]	Days				
	0	4	8	30	60
Pc 3[b]	9.7×10^8	3.1×10^8	1.7×10^8	7.2×10^7	6.6×10^4
Lp 1 + Pc 3[b]	1.9×10^9	9.8×10^8	5.6×10^8	7.7×10^7	3.2×10^4
Lp 4 + Pc 3[b]	1.6×10^9	7.2×10^8	6.0×10^8	2.5×10^7	2.2×10^5
Lp 1[b]	9.5×10^8	8.6×10^8	7.1×10^8	1.3×10^8	3.0×10^5
Lp 4[b]	6.6×10^8	1.0×10^9	6.8×10^8	9.6×10^6	3.6×10^4

[a] Lp = Lactobacillus plantarum; Pc = Pediococcus cerevisiae

[b] Lot 5

TABLE 15. -- Analysis of Variance of data from the first hedonic evaluation of the flavor of a commercial product (Y) and three pure culture fermented samples.

Judges	Samples				Totals
	Y	Pc 3	Lp 4	Lp 4 + Pc 3	
1	7	6	8	6	27
2	4	7	5	4	20
3	9	8	9	8	34
4	7	6	5	6	24
5	6	4	4	3	17
6	8	4	5	7	24
7	7	6	5	6	24
8	7	4	5	3	19
9	8	4	4	4	20
10	4	2	4	8	18
11	8	5	4	6	23
12	2	3	5	6	16
13	6	7	6	6	25
14	8	5	5	6	24
15	3	8	8	5	24
16	8	5	8	5	26
17	4	6	6	5	21
18	5	5	7	6	23
19	8	4	4	4	20
Sums	119	99	107	104	429
Means	6.2	5.2	5.6	5.4	

Correction factor (C. F.) = 2421.59

Total sum of squares (σ_T^2) = 215.41

Sum samples squares (σ_F^2) = 11.41

Sum judges squared (σ_J^2) = 77.16

Residual error (σ_R^2) = 126.84

TABLE 16. -- Analysis of Variance of data from the second hedonic evaluation of the flavor of a commercial product (Y) and three pure culture fermented samples.

Judges	Samples				Totals
	Lp 4 + Pc 3	Pc 3	Y	Lp 4	
1	2	7	8	5	22
2	7	6	8	7	28
3	4	4	6	5	19
4	6	7	7	7	27
5	6	7	6	6	25
6	4	3	5	7	19
7	7	8	4	8	27
8	4	5	6	8	23
9	9	8	3	6	26
10	7	3	7	7	24
11	7	6	7	5	25
12	7	6	7	5	25
13	6	5	7	5	23
14	5	7	5	6	23
15	7	7	8	6	28
16	4	3	8	6	21
17	5	6	7	6	24
18	8	7	7	7	29
19	8	4	2	5	19
20	9	8	6	9	32
Sums	122	117	124	126	489
Means	6.1	5.8	6.2	6.3	

Correction factor (C.F.) = 2989.01

Total sum of squares (σ_T^2) = 201.99

Sum samples squared (σ_F^2) = 2.24

Sum judges squared (σ_J^2) = 58.24

Residual error (σ_R^2) = 141.51