CONTROLLED LACTIC ACID FERMENTATION OF VEGETABLES

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1. SUMMARY

Fermentation of vegetables as a method of preservation is a process that is difficult to control because of naturally occurring microorganisms, heterogeneous botanical structures and the composition of the raw vegetable material. Whether products of high and consistent quality can be obtained by industrial vegetable fermentation will depend on how well this process can be controlled. This paper describes physical, chemical and biological parameters such as temperature; pressure; modified atmosphere; pH; salt- and sugar concentration and type of starter culture to control the fermentation. The strategies for fermentation control are discussed from an ecological point of view, emphasizing the influence of the control factors on the microorganisms involved in the fermentation process.

2. INTRODUCTION

The preservation of vegetables by fermentation was recognized before recorded history. Although the exact origins are unknown, there are Egyptian mural paintings from 1000 - 1500 B.C. showing the ensilage of grain (Woodford, 1985). It is believed that during the construction of the Great Wall of China in the 3rd century B.C., a fermented mixture of vegetables was given to the labourers (Pederson, 1979). Since lactic acid bacteria are naturally present on most living plants (Etchells et al., 1961; Mundt, 1970), spontaneous lactic acid fermentation occurs when vegetables are brined. The lactic acid fermentation results in products with a longer shelf life compared to fresh vegetables. The capability to store vegetables and protect them from spoilage made fermentation important as a preservation method for vegetables and silage. Although a number of other vegetable preservation methods are applied today, fermentation is still important as a method to bulk-store large volumes because of its low energy costs. Besides being preserved, fermented products are known to have remained palatable. Nowadays lactic fermentation is also used to improve the sensory product quality, and this is another reason why it remains an important process in modern and developing countries. It deserves, however, a wider application provided the fermentation process can be properly controlled.

Large volumes and many varieties of plant materials have been subjected to fermentation and then used for human and animal consumption. The microorganisms responsible for the fermentation also differ widely. The most important fermented vegetables are pickles, sauerkraut and olives. Besides, many forage crops like alfalfa, corn and grass are ensiled. Each plant provides a unique environment in terms of naturally occurring microorganisms, and the structure and composition of the substrate. These factors highly influence the sequential growth of fermentation microorganisms after the plant material is harvested and prepared for fermentation (Daeschel et al., 1987). The majority of the vegetable products are fermented by lactic acid bacteria. Yeast and molds may also be involved, but to a smaller extent. Although relatively few species of lactic acid bacteria are responsible for the fermentation, the process is very complex from an ecological point of view. Microbiological research is therefore essential if improvements in vegetable fermentation are to be made.

The success of industrial vegetable fermentations will to a large extent depend on proper control of the process in order to obtain products of high and consistent quality. However, compared to other types of fermentation, fermentation of vegetables is difficult to control primarily because of the presence of naturally occurring microorganisms on the raw materials, plant structure, the differences in the availability of nutrients and the presence of natural plant antagonists. The aim of this paper is to review physical, chemical and biological parameters involved in the control of lactic acid fermentation of vegetables.

3. FERMENTATION PROCESSES

From a mechanical point of view, the transformation of any plant raw material to a lactic acid-fermented product is a simple operation. However, from a biochemical and microbiological viewpoint, the fermentation is enveloped in an array of complexities. A generalized flow chart for lactic acid fermentation of cabbage, cucumbers, olives and root crops is presented in Figure 1.

Although the fermentation processes differ according to the raw materials and the products, lactic acid fermentation of vegetables can be discussed in general terms. Environmental factors important in the fermentation of vegetables consist essentially of a high quality raw material, the establishment of anaerobic conditions, a suitable salt concentration, proper temperature, hygiene and the presence of desirable lactic acid bacteria.

Cabbage	Cucumbers	Olives	Root crops
Trim line	Wash	Debitter	Wash/trim
Shredd	Tank	Barrel/tank	Slice/shredd
Salt	Brine	Fermentation	Tank
Fermentation	Acidify	Store	Brine
Sauerkraut	Purge	Product	Starter culture
	Buffer		Fermentation
	(Starter culture)		Store
	Fermentation		Product
	Store		
	Further process		

Fig. 1 Flow charts for lactic acid fermentation of vegetables

3.1. Pre-treatment of the vegetable raw material

A careful examination of the raw materials is important to sort out those which are diseased, defect, injured or moldy. Trimming and cleaning are essential for removing soil and reducing the number of extraneous microorganisms. For cabbage this is done by mechanically removing the outer leaves and the core. For other vegetables, water, sometimes chlorinated or warm, and brushes are used to optimize cleaning. Olives are bitter in their natural state because the outer coating contains a glucoside, oleuropein. Most of the bitterness can be removed by soaking the fruit in lye. Besides debittering, this step also reduces the risk of formation of hydrolysis products of oleuropein which are inhibitory against lactic acid bacteria and thereby influence the fermentation (Fleming et al., 1973b). Cabbage and root crops are shredded and sliced prior to fermentation, while cucumbers and olives are fermented whole.

3.2. Salt additions

The next main step in the process is addition of salt (NaCl) or brine. In the production of sauerkraut, dry salt is added to the shredded cabbage, while a brine is added to cucumbers, olives and root crops. The final salt concentration varies with the product in question. Root crops are fermented in a brine containing about 1.5 % of NaCl (Andersson, 1984). According to the definition of sauerkraut, the concentration of salt must not be less than 2 %, and not higher than 3 %. Fleming (1984) divided lactic acid fermented cucumbers into three groups with respect to salt concentration. Firstly, "overnight dill" pickles, which are fermented in 2-4 % NaCl; secondly, "genuine dill" pickles, which are fermented in 4-5 % NaCl; and thirdly "salt-stock" cucumbers, which are fermented in a brine containing 5-8 % NaCl followed by an increase of the NaCl concentration to 10-16 %. Salt plays a very important role in lactic acid fermentation of vegetables and sufficient amounts must be added to extract from the plant cells the nutrients required to support growth of the lactic acid bacteria. Salt also serves to inhibit the growth of undesirable microorganisms and serve as a flavor ingredient in the final product (Stamer, 1983).

3.3. The fermentation

Compared to other microorganisms, the initial number of lactic acid bacteria on the plant is small. A spontaneous fermentation is therefore a process in which the lactic acid bacteria become the predominating bacterial flora (Daeschel et al., 1987). Thus, the course of a spontaneous fermentation process is largely unpredictable because of diversities in the initial microbial flora and in the chemical and physical factors that influence the growth of different species. Lactic acid bacteria associated with plants and which generally are responsible for vegetable fermentations belong to the species Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum and Pediococcus pentosaceus. L. mesenteroides predominates during the initial stage of the fermentation, while the other species are involved in the terminal stages of the fermentation (Pederson, 1979; Stamer et al., 1971; Stamer, 1975). In current commercial practice, fermentation of cabbages is carried out by naturally occurring lactic acid bacteria which are initially present on the cabbage leaves. Such uncontrolled fermentation leads to variations in the product quality. The main reason why sauerkraut is still produced by spontaneous fermentation is that man has not yet succeeded in repeating this kind of fermentation course by the use of starter cultures. The order of succession and the ratio of hetero- and homofermentative lactic acid bacteria necessary to obtain a superior sauerkraut is unknown. Fleming et al. (1988b) characterized the sauerkraut fermentation as consisting of two distinct phases. The first is called gaseous owing to rapid CO_2 and acid production by heterofermentative lactic acid bacteria; the second is called nongaseous owing to the growth of nongas-forming lactobacilli. A spontaneous fermentation of cucumbers has been categorized as having four stages (Fleming, 1982): initiation, primary fermentation, secondary fermentation, and post-fermentation (Table 1). This reflects the different types of microorganisms involved and stresses the view that lactic acid fermentation follows a complex microbial ecology course. Similar microbial sequences have been shown for lactic acid fermentation of root crops (Andersson, 1984).

Table 1. Sequence of microbial types during natural fermentation of brined vegetables [a]

Stage	Prevalent microorganisms
Initiation	Various Gram-positive and Gram-negative bacteria
Primary fermentation	Lactic acid bacteria, yeasts
Secondary fermentation	Yeasts
Post-fermentation	Aerobic: surface growth of oxidative yeasts, molds and bacteria Anaerobic: none

[a] From Fleming (1982)

3.4. Strategies to control the fermentation

In plant fermentations, control procedures are necessary in order to prevent spoilage and to provide products of a consistently high quality. As stated by Daeschel et al. (1987), it is unrealistic to assume that a pure culture fermentation can be achieved without a sterilization step. From a practical point of view the approach is controlled fermentation where one or more cultures of known species cause the desirable fermentation. Growth of microorganisms other than the desired species may occur, but their effect on the final product should be inconsequential. The strategies to achieve such a controlled fermentation include physical, chemical and biological mechanisms (Table 2).

Table 2. Strategies to control lactic acid fermentation of vegetables

Control mechanism	Factor
Physical	Fermentation vessel Oxygen exchange
Chemical	Anaerobisis Acids Buffers Salt
Biological	Starter cultures

4. PHYSICAL CONTROL FACTORS

4.1. Fermentation vessel

Cucumber pickles have traditionally been fermented in open-top, wooden vessels ranging in size from approximately 8000 to 32000 liters. Tanks are typically left outside unsheltered from the environment. The sunlight (UV radiation) striking the surface of the brine prevents the growth of oxidative spoilage microorganisms. In addition, the open top allows the escape of CO_2 generated during fermentation. Problems that exist with an open-top tank design include: brine evaporation, rainwater accumulation and contamination with dirt, dust and insects. Numerous attempts have been made to develop enclosure devices for cucumber tanks, but none have proven to be commercially feasible. The major impediment to adopting a closed system was that it did not provide an escape for CO_2 evolved during fermentation. Excessive CO_2 accumulation during fermentation can result in physical disruption of the cucumber tissue due to gas pressure (Fleming et al., 1973a). With the introduction of N_2 purging (Fleming et al., 1975) of cucumber brines to dissipate CO_2 produced during fermentation, it is now technically feasible to consider a closed-top system. Recently, several prototype, closed-top, anaerobic brining tanks have been developed and tested (Humphries and Fleming, 1986; Fleming et al., 1988a). Some advantages that closed tanks may provide include: reduced salt usage and disposal problems, improved and more uniform flavor and color, the possibility of new products and processes, and improved sanitation.

In contrast to cucumber pickles, sauerkraut is fermented indoors in tanks that are maintained anaerobically. The tank is covered with plastic sheeting upon which water is placed, providing a weighted, air-tight seal against the tank wall. Anaerobiosis is necessary to prevent oxidation of the sauerkraut and the growth of aerobic spoilage microorganisms. During the initial gaseous stage, the sauerkraut mass is prone to expansion or "heaving" due to CO_2

entrapped within the sauerkraut. This can lead to deterioration of the sauerkraut if the expanding kraut causes a breach in the anaerobic seal. A model sauerkraut fermentor has been developed (Fleming et al., 1988b) to explore new technologies to eliminate the sauerkraut "heaving" problem. N_2 purging of experimental sauerkraut fermentations was shown to provide a means of brine circulation and removal of the CO_2 responsible for "heaving".

4.2. Oxygen exchange technology

Fleming et al. (1980) observed that exchange of the internal gas of fresh cucumbers with pure O_2 resulted in brined cucumbers developing a fully cured appearance within a few days, as compared with several months for cucumbers not exposed to O_2. The O_2-exchanged cucumbers were less susceptible to bloater damage (ruptured tissue) than nonexchanged cucumbers during fermentation. Fleming et al. (1980) postulated that the O_2 present in cucumbers is rapidly consumed with concurrent production of CO_2 due to respiration when the cucumbers are submerged in brine. The CO_2, having a much greater solubility than the O_2 it replaced, is believed to dissolve in the tissue with a subsequent vacuum being formed. The vacuum is relieved by brine uptake into cucumbers. Corey et al. (1983) confirmed that a partial vacuum occurs in O_2-exchanged, brined cucumbers. Daeschel and Fleming (1983) showed that liquid enters the cucumber through epidermal regions of greatest stomatal density which are near placental tissue. It was also shown that lactic acid bacteria can enter and grow within cucumbers after they are brined (Daeschel and Fleming, 1981). Exchange of the natural gases of cucumbers with O_2 prior to brining significantly increased the entrance and subsequent numbers of bacteria within the fruit. It was proposed that the bacteria can enter the cucumbers through the stomata of the fruit epidermis and that movement into the cucumber was due to brine uptake to relieve the internal vacuum. In a subsequent study (Daeschel et al., 1985a), it was shown that yeasts are unable to enter brined cucumbers even when the cucumbers had been O_2-exchanged prior to brining. Yeasts appeared to be physically excluded from the cucumber interior because of their greater size.

There are several practical implications that can be derived from the studies discussed previously. For example, O_2-exchanged cucumbers rapidly obtain a fully cured appearance. This is an important trait for hamburger dill slices. It takes several months for a fully cured appearance to be reached with traditionally brined cucumbers. Thus, O_2-exchanged technology may be used to greatly reduce processing time. O_2-exchange could also conceivably be used to speed up the fermentation time. The fermentation is, to a large extent, dependent upon the diffusion of fermentable sugars from the cucumber out into the brine where the bacteria can ferment them. Bringing the bacteria into the cucumber by O_2-exchange may enhance the fermentation rate, since sugar diffusion may no longer be a rate-limiting step. These ideas, however, need to be tempered with a note of caution. It is also conceivable that O_2-exchange may at the same time draw into the cucumber undesirable microorganisms which could cause spoilage.

5. CHEMICAL CONTROL FACTORS

5.1. Anaerobisis

In most, if not all, lactic acid fermentations of vegetables, it is necessary to exclude oxygen to provide anaerobiosis. This will inhibit the growth of aerobic spoilage microorganisms, such as bacteria, molds and oxidative yeasts, and stimulate the activity of the lactic bacterial flora. From a practical point of view, anaerobiosis can be self-achieved via the respiratory action of the vegetables and the indigenous micobial flora (Stamer, 1983). Regarding shredded and sliced vegetables, oxygen can be removed by carefully pressing the vegetables in the fermentation vessel. Cucumbers can be purged using either air or N_2 (Fleming et al., 1975). A continuous flushing of CO_2 or N_2 has also been sucessfully used in the fermentation of vegetable cocktails (Zetelaki-Horvath and Andersson, 1986; Andersson, unpublished data). It is well documented that CO_2 stimulates the growth of lactic acid bacteria but inhibits most of the Gram negative bacteria. Anaerobic conditions will also prevent oxidation reactions of the vegetables.

5.2. Acids and buffers

Fleming (1984) showed that initial acidification of the brine inhibited acid sensitive bacteria and favored the growth of lactic acid bacteria. Before inoculation with a starter culture, a buffer such as sodium acetate or calcium acetate was added in order to neutralize the acid. Addition of a buffer made the initial pH favorable to growth of the desired culture of lactic acid bacteria and ensured the fermentation of all sugars. Glucose, fructose and sucrose are the primary plant carbohydrates that are fermented by the lactic acid bacteria. More sugar than necessary is present in the vegetables to produce the acids required for preservation. But in order to avoid secondary fermentations (Table 1), it is important to ascertain that the lactic acid bacteria are able to ferment all sugar. Since the bacterial cultures may be inhibited by low pH and some vegetables contain as much as 10 % (w/w) of fermentable sugars, additional buffering prolongs the activity of the lactic acid bacteria, which in turn reduces the risk of secondary fermentations, e.g. by yeasts.

5.3. Salt

As mentioned previously, NaCl must be added to withdraw water and nutrients from the plant materials before lactic acid fermentation can take place. Furthermore, NaCl inhibits spoiling microorganisms and reduces the effects of softening enzymes. At the same time, however, salt affects the activity of the lactic acid bacteria. Pederson (1979) showed that about 10 % NaCl in the brine is the upper limit for fermentation of vegetables and that the growth of lactic acid bacteria is greatly retarded even below this concentration. Leuconostoc mesenteroides is highly influenced in the range from 1 to 3.5 %, while Pediococcus pentosaceus can withstand higher

NaCl concentrations. Since the initial salt concentration used in pickle fermentations is 2 to 3-fold that used in sauerkraut and root crop fermentations, the microbiology of the fermentations differs.

6. STARTER CULTURES

As previously discussed, the lactic acid fermentation of vegetables is based upon the activity of an indigenous lactic acid bacterial flora. The lactic acid bacteria necessary for a spontaneous fermentation can be introduced from the plant raw material, processing equipment and from the recycled fermented brine. Regardless of the source, environmental conditions must be favorable to the desired microorganisms and unfavorable to others. Although early studies have been made of pure culture inoculation of cabbage, cucumbers and olives, the commercial use of starter cultures is limited to the fermentation of cucumbers.

Some of the factors which may account for the limited use of starter cultures have been summarized by Daeschel and Fleming (1984). The most important factor is probably that the microbial process involved in the lactic acid fermentation of vegetables is too complex and poorly understood to allow its imitation in starter cultures. For this reason it is not only important to characterize the microorganisms involved in the fermentation qualitatively, but also to establish the ratios between the species and their activity. Compared to other commodities, such as milk and meat, the environmental influence on the fermentation of vegetables is much more diverse and complex. This makes the development of a starter culture for lactic acid fermentation of vegetables very challenging. It is reasonable to assume that a tailor-made starter culture for vegetables will contain more than one lactic acid bacterial specie. Characteristics desired in starter culture development for vegetable fermentation have previously been reviewed by Fleming and McFeeters (1981), Daeschel and Fleming (1984), Fleming et al. (1985) and Daeschel et al. (1987).

6.1. Predominance of growth

To become predominant the starter culture has to be highly competitive against other microorganisms including other lactic acid bacteria, Gram positive and Gram negative spoilage bacteria, yeasts, and molds. Its ability to become the predominating flora will depend on rapid growth and acid production. Among the lactic acid bacteria, Leuconostoc mesenteroides grows rapidly and therefore normally initiates the lactic acid fermentation. However, L. mesenteroides has poor acid- and salt tolerance and will in time be replaced by Lactobacillus brevis, Pediococcus pentosaceus and Lactobacillus plantarum, often in this order.

The optimum temperature ranges for the starter cultures will highly influence the growth rates. L. mesenteroides grows optimally at a temperature ranging from 20 to 30 C, while the other lactic acid bacteria mentioned above prefer 30 to 35 C. When the vegetables are fermented outdoors, the ambient temperature varies with the time of year and geographical location. However, cultures with optimum growth capabilities at about 20 C could be especially useful for vegetable fermentations in cooler climates and for obtaining flavored products.

Bacteriocin-producing starter cultures may have enhanced capabilities of predominating the fermentation. Bacteriocins from lactic acid bacteria are antagonistic primarily against other lactic acid bacteria. Therefore, the production of bacteriocin can be a key factor in the inhibition by the starter culture of the growth of other, unwanted, lactic acid bacteria originating from the plant itself. Bacteriocins from lactic acid bacteria have recently been reviewed by Klaenhammer (1988), and in our laboratories we are currently evaluating the potential of bacteriocins from Pediococcus pentosaceus (Daeschel and Klaenhammer, 1985) and L. plantarum (Daeschel et al., 1986; Andersson et al. 1988a) in controlling the microflora in vegetable fermentations.

6.2. Metabolic properties

A complete and rapid conversion of the fermentable sugars to acids and stable chemical products is desired. This is obtained by lactic acid bacteria which are able to utilize primarily glucose, fructose and sucrose. Homofermentative bacteria produce only lactic acid, while heterofermentative strains in addition produce products such as acetic acid, mannitol, CO_2, dextran, and ethanol. A homofermentative starter culture is preferable in the fermentation of cucumbers but not for other vegetables, since a heterofermentation may be preferred to impart a desired flavor. The type of acids produced influence the fermentation not only by lowering the pH but also by having different antagonistic properties. Acetic acid is more inhibitory than lactic acid against yeast.

Malate, an organic acid found in cucumbers can be decarboxylated by lactic acid bacteria. This is undesirable because of CO_2 produced. Daeschel et al. (1984) have isolated mutant strains of Lactobacillus plantarum no longer able to decarboxylate malate. These strains are being developed for commercial use (Daeschel et al., 1985b).

6.3. Improvement of nutritional and sensory properties

Since the time of Metchnikoff (1908), fermented products have had an image of possessing almost magical health-giving properties. However, the knowledge of how lactic acid fermentation influences the nutritional value of products is poor and many of the nutritional aspects of fermented products still have to be subjected to rigorous scientific investigation. Information is available regarding vitamins (Fleming and McFeeters, 1981), nitrate and nitrite (Andersson, 1985), biogenic amines (Andersson, 1988) and the fibre content (McFeeters, 1985; Andersson et al., 1988b) in lactic acid-fermented vegetables.

There is an increased interest regarding the probiotic effects of the lactic acid bacteria themselves. Lidbeck et al. (1987) reported that when Lactobacillus acidophilus was given to humans as a liquid fermented milk product the number of Escherichia coli, Klebsiella oxytoca and Candida albicans in their intestinal microflora decreased. The adhesion and antimicrobial effect of lactic acid bacteria have so far only been studied in a limited number of strains, but it is realistic to believe that such nutritional factors will be important in the future selection of starter cultures for lactic acid fermentation of vegetables.

When digested in a meal, lactic acid fermented vegetables have been shown to increase the bioavailability of iron (Andersson et al. unpublished data). Compared to a non-fermented control, lactic acid-fermented carrots increased the uptake of iron 2-fold from an otherwise identical meal. The reason for the stimulating effect has not been made clear and further studies are needed.

As mentioned previously, the original purpose of lactic acid fermentation of vegetables, preservation, has been expanded to include the improvement of the sensory properties of vegetable food. This means that it will be even more important in the future to understand the influence of the starter culture on the sensory quality. The type of acids produced, and their relative proportions, is of course important, as well as the production of other flavor compounds by the lactic acid bacteria. It might very well be the case that a starter culture consists of a mixture of lactic acid bacteria and other microorganisms, where some strains ensure the preservation properties and some other strains primarily produce flavor compounds.

7. FUTURE DIRECTIONS

Historically, fermented vegetables have played an important role in providing a safe, nutritious, preserved food commodity. Sauerkraut was routinely part of the food supply of the sailing ships used in exploring the New World during the 16th and 17th centuries. It was observed then that scurvy was prevented when sauerkraut was included in the sailors' diet. Since that time and with the introduction of new preservation techniques (freezing, canning, etc.), fermentation as a preservation method has become less commonplace. However, fermentation remains an important technology because it provides for the production of commodities with unique, desirable organoleptic traits, it is a method requiring low energy, and allows the processing of fruits and vegetables to be extended over a longer period of time.

What does the future hold for fermented vegetables? The key to enhancing the quality, reducing the spoilage, and reducing the production costs of fermented vegetables lies in controlling the fermentation process. Successful control of the process will be dependent on the integration of physical, chemical, and biological factors. Anaerobic fermentation tanks, O_2-exchange technology, purging of CO_2 from brines, development of starter cultures with specific traits, and the use of calcium chloride to improve textural qualities are all examples of fermentation control that have been developed in the last 20 years. The new "biotechnology" promises to provide the tools for further improvements, especially in the area of starter culture development and perhaps in the introduction of new vegetable cultivars specially designed for fermentation.

REFERENCES

Andersson, R. 1984. Characteristics of the bacterial flora isolated during spontaneous lactic acid fermentation of carrots and red beets. Lebensm.-Wiss. u.-Technol. 17:282-286.

Andersson, R. 1985. Nitrate reduction during fermentation by Gram-negative bacterial activity in carrots. Int. J. Food Microb. 2:219-225.

Andersson, R. 1988. Biogenic amines in lactic acid fermented vegetables. Lebensm.-Wiss. u.-Technol. (in press).

Andersson, R.E., Daeschel, M.A. and Hassan, H.M. 1988a. Antibacterial activity of plantaricin SIK-83, a bacteriocin produced by Lactobacillus plantarum. Biochimie 70:381-390.

Andersson, R.E., Eriksson, C.E., Salomonsson, A.-C. and Theander, O. 1988b. Lactic acid fermentation of non-stored and stored carrots - Chemical, microbial and sensory evaluation of products. Submitted for publication.

Corey, K.A., Pharr, D.M. and Fleming, H.P. 1983. Pressure changes in oxygen-exchanged, brined cucumbers. J. Amer. Soc. Hort. Sci. 108:61-65.

Daeschel, M.A., Andersson, R.E. and Fleming, H.P. 1987. Microbial ecology of fermenting plant materials. FEMS Microbiology Rev. 46:357-367.

Daeschel, M.A. and Fleming, H.P. 1981. Entrance and growth of lactic acid bacteria in gas-exchanged, brined cucumbers. Appl. Environ. Microbiol. 42:1111-1118.

Daeschel, M.A. and Fleming, H.P. 1983. Rapid and specific staining for routes of liquid entry into cucumber fruit. J. Amer. Soc. Hort. Sci. 108:481-483.

Daeschel, M.A. and Fleming, H.P. 1984. Selection of lactic acid bacteria for use in vegetable fermentations. Food Microbiol. 1:303-313.

Daeschel, M.A., Fleming, H.P. and Potts, E.A. 1985a. Compartmentalization of lactic acid bacteria and yeasts in the fermentation of brined cucumbers. Food Microbiol. 2:77-84.

Daeschel, M.A. and Klaenhammer, T.R. 1985. Association of a 13.6-megadalton plasmid in Pediococcus pentosaceus with bacteriocin activity. Appl. Environ. Microbiol. 50:1538-1541.

Daeschel, M.A., McFeeters, R.F. and Fleming, H.P. 1985b. Modification of lactic acid bacteria for cucumber fermentations: Elimination of carbon dioxide production from malate, in Developments in Industrial Microbiology, Vol. 26, pp. 339-346, Society for Industrial Microbiology, Arlington, VA.

Daeschel, M.A., McFeeters, R.F., Fleming, H.P., Klaenhammer, T.R. and Sanozky, R.B. 1984. Mutation and selection of Lactobacillus plantarum strains that do not produce carbon dioxide from malate. Appl. Environ. Microbiol. 47:419-420.

Daeschel, M.A., McKenney, M.C. and McDonald, L.C. 1986. Characterization of a bacteriocin from Lactobacillus plantarum. Am. Soc. Microbiol., Prog. Abstr. 86,133.

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

Fleming, 1982. Fermented vegetables, in Economic Microbiology. Fermented Foods (Rose, A.H., Ed.), pp. 227-258, Academic Press, New York.

Fleming, H.P. 1984. Developments in cucumber fermentation. J. Chem. Tech. Biotechnol. 34B:241-252.

Fleming, H.P., Etchells, J.L., Thompson, R.L. and Bell, T.A. 1975. Purging of CO_2 from cucumber brines to reduce bloater damage. J. Food Sci. 40:1304-1310.

Fleming, H.P. and McFeeters, R.F. 1981. Use of microbial cultures: vegetable products. Food Technol.35:84-88.

Fleming, H.P., McFeeters, R.F. and Daeschel, M.A. 1985. The lactobacilli, pediococci, and leuconostocs: vegetable products, in Bacterial starter cultures for foods, (Gilliland, S.E., Ed.), pp.97-118, CRC Press, Boca Raton, Florida.

Fleming, H.P., McFeeters, R.F., Daeschel, M.A., Humphries, E.G. and Thompson, R.L. 1988a. Fermentation of cucumbers in anaerobic tanks. J. Food Sci. 53:127-133.

Fleming, H.P., McFeeters, R.F. and Humphries, E.G. 1988b. A fermentor for study of sauerkraut fermentation. Biotech. Bioeng. 31:189-197.

Fleming, H.P., Pharr, D.M. and Thompson, R.L. 1980. Brining properties of cucumbers exposed to pure oxygen before brining. J. Food Sci. 45:1578-1582.

Fleming, H.P., Thompson, R.L., Etchells, J.L., Kelling, R.E. and Bell, T.A. 1973a. Carbon dioxide production in the fermentation of brined cucumbers. J. Food Sci. 38:504-506.

Fleming, H.P., Walter, W.M. and Etchells, J.L. 1973b. Antimicrobial properties of oleuropin and products of its hydrolysis from green olives. Appl. Microbiol. 26:777-782.

Humphries, E.G. and Fleming, H.P. 1986. Closed-top fermentation tanks for cucumbers. Trans. ASAE 2:45-48.

Klaenhammer, T.R. 1988. Bacteriocins of lactic acid bacteria. Biochimie (in press).

Lidbeck, A., Gustafsson, J.-Å. and Nord, C.E. 1987. Impact of Lactobacillus acidophilus supplements on the human oropharyngeal and intestinal microflora. FEMS Microbial Rev. 46:P72.

McFeeters, R.F. 1985. Changes in pectin and cellulose during processing, in Chemical changes in food during processing, (Richardson, T.R. and Finley, J.W., Eds.), pp. 347-372, Avi Publishing Company, Westport, CT.

Mundt, J.O. 1970. Lactic acid bacteria associated with raw plant food material. J. Milk Food Technol. 33:550-553.

Pederson, C.S. 1979. Microbiology of food fermentations, 2nd edition, Avi, Westport,CI.

Metchnikoff, E. 1908. The prolongation of life, G.P. Putnam's and Sons, New York.

Stamer, J.R. 1975. Recent developments in the fermentation of sauerkraut, in Lactic acid bacteria in beverages and food (Carr, J.G., Cutting, C.V. and Whiting, G.C., Eds.), pp.267-281. Academic Press, New York.

Stamer, J.R. 1983. Lactic acid fermentation of cabbage and cucumbers, in Biotechnology, Vol.5 (Rehm, H.-J. and Reed, G., Eds.), pp.365-378, Verlag Chemie, Weinheim.

Stamer, J.R., Stoyola, B.O. and Dunckel, B.A. 1971. Growth rates and fermentation patterns of lactic acid bacteria associated with the sauerkraut fermentation. J. Milk Food Technol. 34:521-525.

Woodford, M.M. 1985. The silage fermentation, in Microbiology of fermented foods, Vol. 2 (Wood, B.J.B., Ed.), pp. 85-112, Elsevier, New York.

Zetelaki-Horvath, K. and Andersson, R. 1986. Fermented vegetable cocktails. Acta Aliment. 15:265-279.

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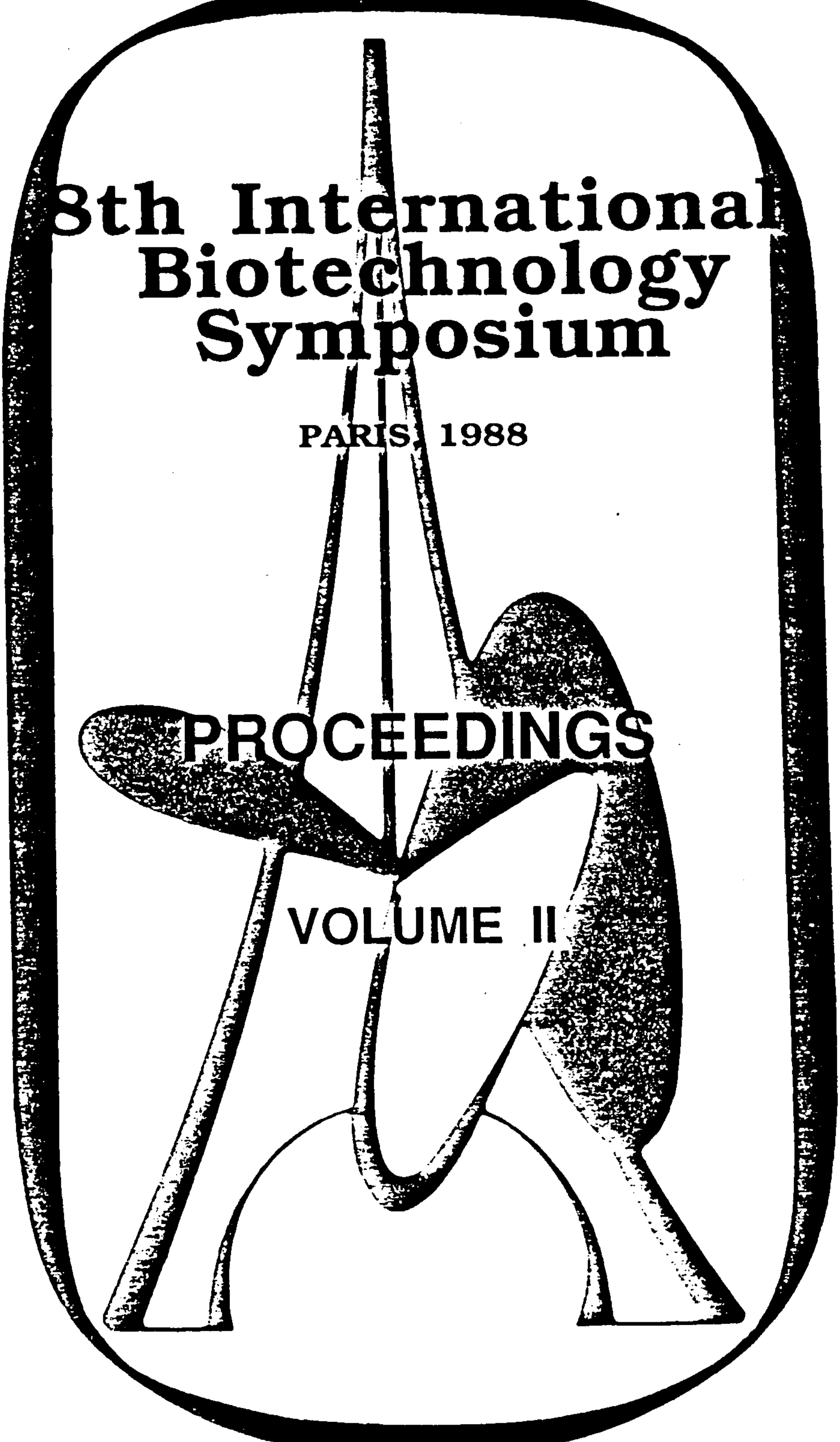
PROCEEDINGS

VOLUME II



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