Softening of Cucumbers during Curing

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Cucumber softening during curing results in large losses to the pickle industry. Disintegration of the tissue is due to the breakdown of the pectic materials in the middle lamella. Pectic enzymes, which catalyze the hydrolysis of pectic materials, occur widely in nature. However, the possible sources of the softening agent(s) are limited to those enzymes which are not inactivated by the acidity and salt content existing in the brine. The aerobic bacilli, once considered to be important spoilage organisms, appear to play a less prominent role in softening than was thought formerly. The probable causes are fungi introduced via heavily contaminated cucumber flowers and the cucumbers (and accessory parts) themselves.

SOFTENING OF CUCUMBERS during curing results in an estimated annual loss of from $500,000 to $750,000 to the industry (*22*). Attempts, on the part of the packer, to eliminate the problem by altering a particular salting procedure have failed. Various aspects of the curing process have been studied in different laboratories for several years and research on the softening problem has been emphasized during the past decade. Cucumber fermentation is a complex process due to the selective action of a continually changing environment. Hence, many species of microorganisms and thus many potential sources of the softening agent are involved.

Normal Fermentation

Practice The main purpose of brining is to preserve the cucumbers until the packer is ready to manufacture them into various types of pickle products. This is a necessity, since the cucumber-growing season lasts only a few weeks, but the packing plant operates throughout the year. It was estimated in 1951 that at least 20% of the annual crop is packed fresh and pasteurized, while the remainder is brined (*24*).

There are many different salting procedures; however, most of the current procedures are based on the same principle. The fresh cucumbers are placed in large wooden vats containing a few inches of brine, and covered with wooden boards to prevent them from floating above the brine surface, and brine of sufficient strength is added to give a final salt concentration of 8 to 10%, after equilibrium between the cucumbers and the brine. Periodically, the salinity is determined and dry salt is added, according to the individual procedure. After about 5 weeks, when the salt concentration has been increased to about 16%, it is held at this strength until the pickles are used (*24*). Some packers add dry salt to the cucumbers while filling the tank (*59*), and add water after the covering boards are put in place. The vats are usually left in the open and the surface is exposed to sunlight, as this reduces the growth of film yeasts on the surface.

Bacteriology of Fermentation During this curing process, soluble nutrients pass from the cucumber into the brine and are then utilized by the salt tolerant bacteria present. An active lactic acid fermentation, lasting for about 6 weeks, results mainly from the growth and activity of the non-gas-producing (homofermentative) *Lactobacillus plantarum* (*23*). Recently, *Pediococcus cerevisiae and Lactobacillus brevis* have also been shown to participate in the fermentation (*6, 9*).

Two types of gaseous fermentations, considered undesirable but not concerned with softening, may occur (*20, 61*). One is brought about by subsurface yeasts producing carbon dioxide while the other is a hydrogen–carbon dioxide fermentation carried out by species of the genus *Aerobacter*. These bacteria thrive in high salt concentrations. Both fermentations cause the "bloater" type of spoilage in which gas pockets are produced inside the cucumbers. The yeasts are mainly responsible,

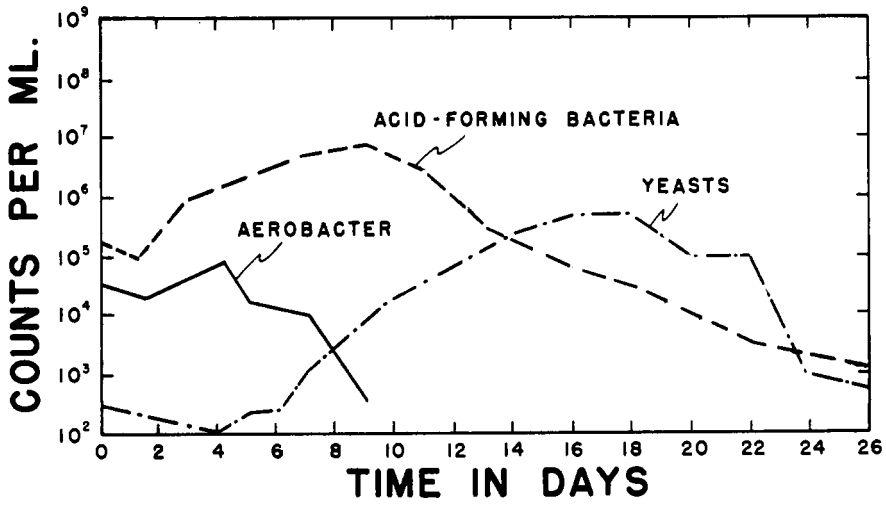

Figure 1. Bacteriological changes observed in a cucumber fermentation started at 10% salt concentration and increased 1.25% per week until 15%. Average brine temperature, 80° F. (27° C.)

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since the *Aerobacter* organisms are very sensitive to the acid produced by the lactobacilli. Jones and coworkers (*37*) have suggested the following control measures: A high salinity should be avoided at the start of the fermentation, and sugar and lactic acid should not be added to the fermentation. The demonstration that the addition of sugar not only failed to increase brine acidity or the rate of acid production but instead increased the percentage of bloaters (*62*) was very significant both from the fundamental and practical viewpoints. Ever since 1899, authorities had been recommending the addition of sugar to salt-stock and dill pickle brine (*1, 8, 29, 31, 38, 40*).

The sequence of bacteriological changes was examined by Etchells and Jones with fermentations starting at 5, 10, and 15% salt (*21*). The data obtained with the fermentation at an initial salt concentration of 10% are reproduced in Figure 1. The salt concentration was increased 1.25% per week until 15% was reached. *Aerobacter* and lactobacilli predominated at the start of the fermentation. After five days, the population of *Aerobacter* decreased rapidly, owing to growth and acid production of the lactic acid bacteria. The latter reached their growth peak at the ninth day and gradually declined. The yeast development began slowly. After a week, there was an increase in population reaching a peak at about 16 to 18 days, and a decline thereafter. The chemical changes which occurred correlated with the type of flora present throughout the fermentation (*36*).

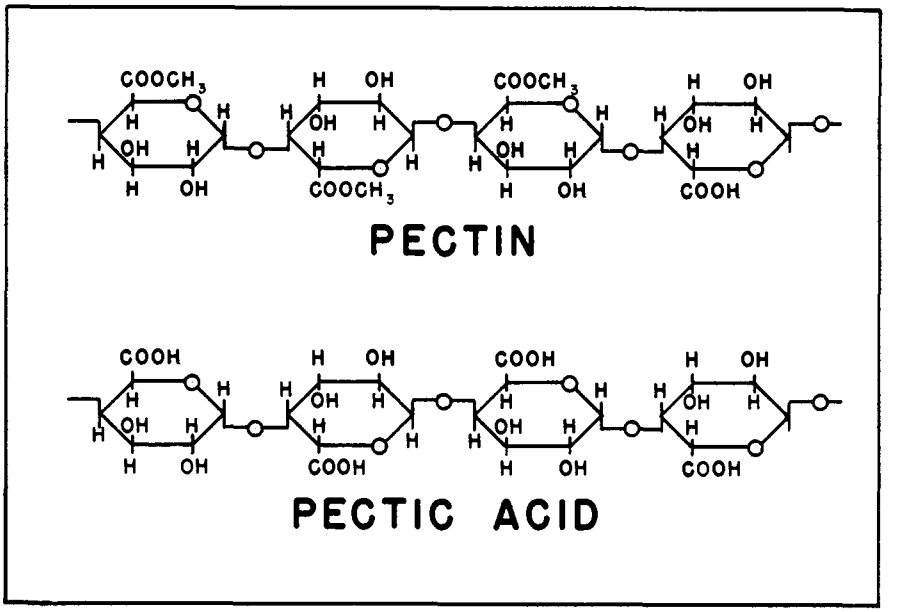

Figure 2. Structure of pectin and pectic acid

Softening

Relationship to Pectin Breakdown

Softening occurs when the pectic material, functioning as cementing agents of the tissue cells, is broken down. Thus, softening is intimately connected with the group of hydrolytic enzymes known as the pectic enzymes.

Pectic substances are essentially long chain polymers of D-galacturonic acid. As shown in Figure 2, the residues are connected by α-1,4-glycosidic linkages. Pectin differs from pectic acid in that about three of every four residues are esterified with methanol. The enzymes responsible for the breakdown of these polymers are the subject of intensive current research throughout the world and ideas concerning them are continually changing.

In Figure 3, we have attempted to give a simple comprehensive summary of current ideas on the subject. The enzymes differ in the substrate attacked, the extent of hydrolysis, and in their mechanism of hydrolysis—i.e., most catalyze a random hydrolysis while terminal hydrolyzing enzymes have been found which remove galacturonic acid residues from the end of the chain. Furthermore, the various enzyme types have different pH optima.

Pectinesterase (PE) removes methoxyl groups from pectin, producing pectic acid. This enzyme has been found in fungi and higher plants (*43*). The pectic acid can then be completely hydrolyzed randomly by mold polygalacturonase, the well-known commercial filtration enzyme (*34*). Tomato polygalacturonase carries out the same reaction (*44*). Yeast polygalacturonase acts in a similar manner, except that it ceases its action after 70% hydrolysis, leaving a mixture of digalacturonic and galacturonic acids (*11, 42*). The random action of these three enzyme preparations results in progressive splits of the large polymer into smaller oligogalacturonic acids which are also attacked but at a lower rate (*11, 45*).

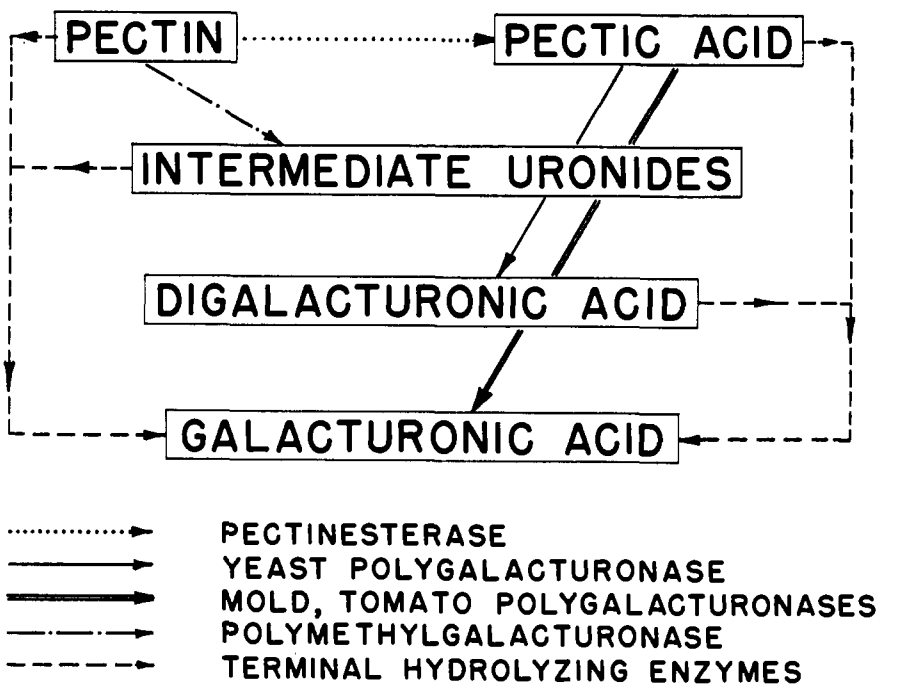

Figure 3. Scheme of pectin and pectic acid decomposition by the pectic enzymes

Pectin itself can be randomly hydrolyzed by polymethylgalacturonase (*56*) and to a very limited extent by ordinary fungal polygalacturonase (*34*) or yeast polygalacturonase (*42*). In the case of polymethylgalacturonase, the reaction ceases after approximately one quarter of the glycosidic bonds have been split. Enzymes similar to polymethylgalacturonase have been found in *Neurospora* (*52*) and *Aspergillus* (*13*). The terminal hydrolyzing enzymes have been worked on only recently. They have been found in several fungi (*7*, *46*, *49*, *55*, *57*).

Evidence of Pectin Disintegration

The first indication that the breakdown of pectic substances was involved in pickle softening was presented by Fabian, Bryan, and Etchells in 1932 (*26*). Microscopic examination of both firm and spoiled pickles showed an absence of what was thought to be pectic material between epidermal and parenchymatous cells in the latter. Further work by Fabian and Johnson (*28*) showed that the following events accompanied spoilage:

A complete loss of ruthenium red stainability.

A twofold increase in soluble pectin (from 0.53 to 1.06% on the dry weight basis).

No change in total pectic materials, measured as calcium pectate (13.0 and 12.9%, respectively, on the dry weight basis).

Apparently, spoilage can occur via the conversion of insoluble pectic material (protopectin) to more soluble forms and smaller molecules without the loss of calcium pectate-forming ability. This change is accompanied by the loss of stainability by ruthenium red. Although this was not determined, the molecular size of the pectic molecules probably decreased during the softening action.

Finally, in 1950, Bell, Etchells, and Jones (*5*) demonstrated that curing brines possess polygalacturonase and pectinesterase activity. Good correlation was observed between the presence of polygalacturonase in the brine and the firmness of the salt-stock. Pectinesterase activity did not appear to be related to softening. Furthermore, when purified mold polygalacturonase was added to cured cucumbers, softening resulted.

Evaluation of Caustive Agents

The determination of the origin of the softening agents has been the most difficult part of research on cucumbers. No doubt it is a complex problem and one that requires careful experimentation. To study the problem accurately, one must consider many possible origins of the softening enzyme: bacteria, yeasts, molds, cucumber and accessory parts, and any combination of these.

Bacteria. Until recent years, the most popular postulation was that pickle spoilage was due to pectic enzyme elaboration by the spore-forming, aerobic, mesophilic bacteria (members of the genus *Bacillus*). When *Bacillus mesentericus fuscus* (*Bacillus subtilis*) was isolated from spoiled brine (*28*), it was shown that the organism grew readily in 9% salt, 0.2% acetic acid, and 0.3% lactic acid (calculated as acetic). Upon addition of the filtrate from this organism (grown in beet molasses medium, pH 6.0) to firm desalted salt-stock pickles, spoilage occurred in 24 hours. Faville and Fabian (*30*) continued this line of investigation and isolated *B. mesentericus fuscus* and *B. vulgatus* (both *B. subtilis*) from spoiled brines. Filtrates of these cultures softened fresh salt stock and in addition could inhibit growth of *L. plantarum*. In 1950, Demain and Fabian (*10*) again isolated *B. subtilus* from spoiled pickle brine and showed that the filtrate from a beet-sugar molasses culture was able to soften desalted pickles.

Despite the attractiveness of this hypothesis, certain facts indicated that bacilli play only a minor role, if any, in pickle softening under natural conditions.

The first indication that pectic enzymes from bacilli might not be the main cause of softening was noted by Fabian and Johnson (*28*). They found that the ability of their bacterial enzyme to soften pickles was inhibited by 2% salt. By growing the culture in the presence of high salt concentrations they could raise the salt tolerance of the enzyme only up to 7%. Softening occurs at much higher salt concentrations, and it is difficult to visualize softening under commercial conditions by their organism. Furthermore, Bell, Etchells, and Jones (*5*) demonstrated that the pectolytic factor in commercial cucumber brines is not inhibited by salt concentrations as high as 21%.

The studies of Nortje and Vaughn (*47*) have cast further doubt on the importance of the aerobic spore formers. These workers isolated bacilli from spoiled brines and tested the activity of filtrates on desalted pickles, using pH control. They showed that only at pH values above 5.7 were the cucumbers softened in the 4-day test period. This is very significant, considering that commercial cucumber fermentations take place between pH 3 and 4. The optimum pH of the *B. subtilis* enzyme was 8.5, while that of *Bacillus pumilis* was 9.4. However, Bell, Etchells, and Jones (*5*) demonstrated that the softening enzyme found in commercial brines had optimal activity in the pH range of 4 to 5. Nortje and Vaughn also demonstrated the instability of the *B. subtilis* pectic enzyme at pH 4.5. In 3 days, 96% of the activity was destroyed.

In a more recent study, Vaughn and coworkers (*60*) concluded that it would be possible for bacilli to cause softening if they were the predominant microbial population, if the pH was 5.5 or above, and if the lactic acid fermentation was slowed down to a point where the pH remained high for several days. However, these conditions are too restricted to account for the major proportion of outbreaks of pickle softening.

Assuming the bacilli to be relatively unimportant, are there any other bacteria to consider? Since most of the soft-rot bacteria produce pectic enzymes with rather high pH optima (*32*, *39*, *48*, *63*) and poor stability at low pH values (*63*), it is doubtful that these organisms can be implicated. Furthermore, they are sensitive to the high salt concentrations found in brine. The obligate anaerobic spore formers (*Clostridia*) have long been known to be potent pectin degraders as exemplified by their use in the anaerobic retting of flax (*51*, *58*). However, favorable growth conditions for these organisms, with respect to both brine pH and salt content, are generally lacking (*25*, *33*). The actinomycetes, some of which produce pectic enzymes (*54*, *57*), represent a relatively unexplored area, as far as softening is concerned. Because they do not grow well under acidic conditions, it is not likely that they play an active role in softening. Nothing appears to be known about their salt tolerance. Yet because of their widespread occurrence in soil, it is desirable that they be studied.

Yeasts. The yeast flora of the commercial cucumber fermentation has been exhaustively studied by Etchells and coworkers (*14*, *15*, *17*, *19*). The yeast flora may be divided into the oxidative film yeasts which produce surface films and the subsurface yeasts responsible for gaseous fermentation. Members of the genera *Debaryomyces*, *Endomycopsis*, *Zygosaccharomyces*, and *Candida* are responsible for film formation, while subsurface yeasts include species of *Torulopsis*, *Brettanomyces*, *Zygosaccharomyces*, *Hansenula*, *Torulaspora*, and *Saccharomyces*. Because yeasts possess high salt and acid tolerances, their production of pectic enzymes, if demonstrated, could be very important.

Etchells and Bell (*15*) showed that several of the above-mentioned film formers (species of *Debaryomyces*, *Endomycopsis*, and *Candida*) could cause deesterification of pectin. Later, these two workers (*3*) found species of *Hansenula*, *Rhodotorula* and *Zygopichia* also to be capable of deesterifying pectin. Luh and Phaff (*41*) found that very few yeasts could clarify a pectin solution. Out of a large number, only *Saccharomyces fragilis* and its imperfect form, *Candida pseudotropicalis*, were active. The clarification of pectin by *S. fragilis* was accompanied by a loss in jellying power but no loss in the ability to form calcium pectate and an alcohol precipitate.

Roelofsen (*53*), who used a different technique to detect pectin-splitting enzymes produced by yeasts, found a greater number of pectolytic species of yeast, belonging to the genera *Candida*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces*. Bell and Etchells (*3*) studied the ability of their brine yeasts and other cultures to destroy the ability of a pectin solution to form a calcium pectate gel. None of the brine yeasts were active. The controls, *S. fragilis* and a strain of *S. cerevisiae* from citrus concentrate, showed activity. As a further check, Etchells and Bell (*16*) added these same yeasts to pasteurized fresh cucumbers in the presence of 0.3% lactic acid and 5 to 6% salt. Yeast growth was followed throughout the experiments. The brine yeasts and *S. cerevisiae* were negative and the *S. fragilis* was positive in pickle-softening activity. Thus the data of Bell and Etchells give no evidence that brine yeasts would be incriminated as a potential source of the salt-stock softening enzyme polygalacturonase.

Pectinesterase itself is not thought to be responsible for softening. However, its presence will accelerate the action of polygalacturonase on pectin (*35*), and the rate of acceleration is directly related to the degree of deesterification of the pectin substrate. Thus, even if brine yeasts are unable to split the glycosidic bonds of pectic materials, those which can elaborate pectinesterase may be important in supplementing a process catalyzed by other agents (*24*).

Molds. Despite the fact that many species of molds are known to be potent producers of pectic enzymes, they have only recently been considered seriously as softening agents. Their aerobic nature would not enable them to develop well under the anaerobic conditions of cucumber fermentation. Until recently the only mention of their action in relation to softening occurred in a publication by Fabian and Faville (*27*). However, these workers concluded that in this case the softening, presumably caused by *Geotrichum candidum* (*Oospora lactis*), occurred after removal of the salt-stock from the vats—i.e., during removal of the salt from the pickles. Thus, here the curing process was not implicated in the softening.

The recent work of Etchells, Bell, and Jones (*18*) implicates molds as the agents of softening during curing. Fungi were found to enter the fermentation via the heavily contaminated cucumber flowers which may remain attached to the fruit after the blooming period. It was shown that brine from vats filled with cucumbers containing a high incidence of flowers (either naturally present or added experimentally) had high polygalacturonase activity and inferior salt stock resulted. When cucumbers were freed from flowers, enzyme activity was low and the pickles were very firm. These authors found that another means of reducing the incidence of softening consisted of draining the original brine from the tanks after 36 hours and replacing it with fresh brine. Over 1000 cultures of molds obtained from the flowers, ovaries, and fruits were isolated and identified. Almost all of the isolates were shown to have pectin-splitting ability. Although molds do not survive long in the unfavorable anaerobic brine environment (except possibly on the brine surface or on the edges of the tanks), they might be able to secrete some pectic enzymes into the brine. The possibility might also be considered that some of these fungi secrete pectic enzymes into the nectar of the flower during blooming, especially under rainy conditions. As most of the mold polygalacturonases have pH optima between 4 and 5, the hydrogen ion concentration of the brine would favor the activity of these pectic enzymes.

Cucumbers and Accessory Parts. Bell, Etchells, and Jones (*4*) have shown that the seeds, leaves, petioles, stems, flowers, and the cucumber itself contain pectinesterase. Furthermore, Bell (*2*) has demonstrated that ripe cucumbers, their seeds, staminate flowers, and pollinated pistillate flowers exhibit pectin-degrading activity at pH 4. The assay was based on the drop in viscosity of a 3% pectin solution. Thus, the demonstration of a pectic enzyme system in the parts mentioned above cannot be disputed.

Discussion

It is evident that there are potentially a number of sources of the enzyme(s) responsible for pickle softening. At present, the most promising possibilities seem to be from molds, the cucumber, and its accessory parts. It would be of interest to compare the activities of these preparations with that of the brine enzyme(s) using both pectin and pectic acid as substrates. Paper chromatography should be particularly useful for such a study. Further comparison of these enzymes by conventional methods of enzymology would enable one to associate the true softening agent with one or more of the possible sources. This general approach to the problem is similar to the one suggested by Pandhi (*50*) in a recent review. One of the major obstacles in such a study would be the very low concentration of pectic enzyme(s) in the brine (*5*). If the recently described pectic acid–gel adsorption method (*12*) for concentration of polygalacturonase could be applied to brine solutions from softened pickles, a study of the enzymes responsible for softening would be facilitated greatly. With the continued cooperation between science and industry, the final solution of the problem should be approached rapidly.

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FERMENTATION YIELDS

Factors Influencing the Production of Polyhydric Alcohols by Osmophilic Yeasts

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Certain osmophilic yeasts produce considerable quantities of glycerol, erythritol, and D-arabitol during normal growth. A culture producing good yields of glycerol and D-arabitol was grown successfully in 5-liter stainless steel fermentors. Satisfactory yields of glycerol and D-arabitol were obtained using a glucose–yeast extract–urea medium, but corn steep liquor could be substituted for yeast extract if higher concentrations of urea were used. Increased rates of aeration decreased the rate of glucose utilization and the yield of ethyl alcohol and increased the glycerol yield, while the yield of D-arabitol was not affected by changes in aeration. Increasing the fermentation temperature, to 37° C., increased the yield of glycerol and the rate of glucose utilization. The initial glucose concentration could be raised to 30% without decreasing the amount of glucose converted to polyhydric alcohols. Ratios of glycerol and D-arabitol produced to glucose metabolized of 0.29 and 0.31 gram per gram, respectively, giving a combined yield of 0.60 gram of polyols per gram of glucose, have been obtained.

Fermentative glycerol production of yeast "steered" with sulfite or alkali has been known for some time. However, the use of these salts increases the cost of the product, both by increasing the cost of materials and by increasing the difficulty of recovery of glycerol, so there is still interest in unsteered fermentative processes for glycerol production.

Several organisms have been found to produce glycerol during normal fermentation (*4, 6, 8*). According to these reports, *Zygosaccharomyces acidifaciens*, a yeast isolated by Nickerson (*7*) from sour wine, possessed the most desirable characteristic—i.e., production of good yields of glycerol, in the absence