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CONTENTS

RELATIONSHIP OF FREEZING PRESERVATION PARAMETERS TO TEXTURE-RELATED STRUCTURAL DAMAGE TO THERMALLY PROCESSED CRUSTACEAN MUSCLE[1]

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ABSTRACT

Selected freezing preservation parameters applied to thermally processed blue crabs and shrimp were evaluated by means of scanning electron microscopy to determine the relationship of the parameters to texture-related structural damage brought about by ice crystal formation and growth. Ice crystal growth and tissue disruption occurs largely during post-freezing frozen storage, the rate of freezing merely setting the pattern of subsequent crystal growth. Damage is more severe with slow (sharp) freezing due to growth of large extracellular ice crystals, causing tissue dehydration and shrinkage and compaction. This also leads to greater drip loss upon thawing. Rapid freezing in "Freon" food freezant or in liquid nitrogen (LN_2) results in less disruptive intracellular crystal formation and growth, the water tending to remain in the tissue upon thawing. Immersion in LN_2 caused noticeable stress-cracking of the tissue, but there was no evidence of significant ice crystal growth during subsequent warming to freezer storage temperatures. Ice crystal growth during $-29°C$ storage was at least one-third slower than at $-10°C$, underscoring the overriding importance of good holding conditions following rapid freezing. It can be concluded that the various fast freezing methods are essentially equally suitable as long as the freezing rate is well within the limit for promoting formation of tiny intracellular ice crystals (i.e. on the order of a few to several minutes). Double freezing of shrimp, a common commercial practice, doesn't appear to result in

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significant damage to tissue over and above that which develops during post-thermal processing frozen storage. It is advisable to upgrade frozen storage systems in the distribution channels to the level of sophistication of modern freezing systems, and to hold product for as short a time at as low a subfreezing temperature as is practical.

INTRODUCTION

Freezing-frozen storage of muscle foods can result in marked textural deterioration. The controllable parameters that can influence quality are mode, temperature and rate of freezing and thawing, frozen storage temperature, and temperature fluctuations during frozen storage, especially at the retail level and in the hands of the purchaser (van der Berg and Lentz 1974; Sanderson-Walker 1975; Sheldon 1975). Recognizing this, concerned industry organizations have collaborated in developing a code of recommended practices for the handling of frozen foods which undergoes periodic revision (Anon. 1975). In recent years freezing processes and especially fast freezing equipment have been engineered to a high level of technical sophistication (Farmakis 1973; Everington 1973; Timbers 1974). However, it is generally recognized that the benefits of rapid freezing of high quality raw material are often undermined by uncontrolled thawing (Everington 1971; Jason 1974) and generally by mishandling in the distribution chain and in the home, and that this is particularly critical with fragile frozen foods such as crustacea (Sheldon 1975).

Shrimp and prawns constitute a sizeable international and national market commodity that is largely distributed in the raw and precooked frozen stages. Other crustacea that are marketed to some extent in the frozen state include crab and lobster species. In the Middle-Atlantic region of the U.S. there is industry interest in the freezing preservation of precooked blue crab (*Callinectes sapidus*) although little is actually done commercially as yet. This is in part due to deterioration in eating quality during frozen storage although recent improvements in methodology have overcome these obstacles to a considerable extent (Ampola and Learson 1972; Webb *et al.* 1976).

In an earlier research note (Giddings and Hill 1976) the feasibility of employing scanning electron microscopy (SEM) to study thermal processing/freezing preservation-connected structural damage to muscle food was demonstrated on cooked-frozen blue crab muscle. Light microscopy and, to some extent transmission electron microscopy have proven useful in following the effects of ice crystal formation and growth

in frozen raw fish tissue (Mills 1975). However, they are not truly suitable for such studies on thermally processed tissue whereas SEM is highly effective and permits the use of "thick" sections (actually, representative pieces of muscle). The major emphasis of that preliminary effort focused on working out and adapting procedures for this specialized SEM application on fully cooked muscle. In this follow-up study sharp freezing at −10 °C and at −29 °C was compared with rapid freezing in "Freon" Food Freezant (E.I. duPont DeNemours and Co., Wilmington, Del., boiling point −30 °C) and in liquid nitrogen (LN_2, boiling point −196 °C) employing both blue crab and shrimp (*Penaeus setiferus*). Two storage temperatures (−10 and −29 °C) and varying storage times were also compared. The two storage tmperatures are well to either side of −18 °C which is believed by many to be the upper limit for acceptable frozen storage of food; −23 to −30 °C being recommended for seafood (Somers 1975; Spiess 1975).

In the case of shrimp, the effect of double freezing was also included. This is a common commercial practice in which frozen "green" (raw) shrimp are received, thawed, cooked and refrozen (Lawler 1969; Anon. 1972). Concern has been expressed that double freezing of fishery products may reduce storage life compared with that of analogous once-frozen products; that is, products prepared from fresh rather than frozen raw material (Connell 1975). On this point, Chinnamma (1973) reported that double frozen raw Indian Ocean prawns exhibited pronounced toughening, elevated drip loss and inferior flavor compared with once-frozen prawns. Ruello (1976) indicates that toughening of prawns can result from (a) freezing and thawing, (b) dehydration during frozen storage, (c) uptake of greater than 4% salt, (d) storage in acidic solutions and (e) prolonged cooking of prefrozen prawns. She notes that many consumers become accustomed to the tougher texture of frozen prawns, preferring it to the "too soft" texture of those freshly caught. Thus it was of interest to determine whether or not differences due to double freezing could be observed in scanning electron micrographs of cooked prefrozen shrimp as compared with those of fresh-cooked, frozen shrimp.

EXPERIMENTAL

Live blue crabs and shrimp were thermally processed within a day of harvest at the North Carolina coast essentially according to the method of Ampola and Learson (1971). Crabs were steam autoclaved for 10 min at 100 °C exclusive of come-up time. Appendages were discarded and body sections (cores) were cleaned and eviscerated. After cooling to 5 °C the meat was frozen in the intact cores. Most of the shrimp was deheaded

and tail sections submerged in a boiling 3% salt-water brine for 4 min in a steam-jacketed kettle, and after cooling in ice water, peeled and deveined. Remaining shrimp were deheaded and sharp frozen raw at −29°C in order to examine the effect of holding in the raw frozen state prior to thermal processing and refreezing (i.e. double freezing). These were held for six weeks at −29°C before thawing overnight, thermal processing as above, and refreezing.

Three modes of freezing were employed: (1) immersion in liquid N_2 in a large dewar, (2) immersion in "Freon" Food Freezant, and (3) sharp freezing at both of the frozen storage temperatures, −10 and −29°C. In the latter case crab cores and shrimp were prewrapped in impermeable film prior to placing in the freezers. LN_2 and Freon frozen samples were similarly wrapped following immersion and were immediately divided into two lots, one held at −10°C and the other at −29°C. During freezing in LN_2 and in "Freon" freezing rates were recorded by inserting a copper-constantan thermocouple wire into the center of the muscle of several shrimp and crab cores. The other end of the wire was attached to a recording device (Newport Laboratories, Inc. Model 810 Digital Thermocouple Indicator/Printer) which printed the center temperature at 2-s intervals. Wired shrimp and crab cores were immersed together with several others in cheesecloth bags which were held submerged until freezant temperature was reached as indicated by the recorder. At zero time (immediately after freezing) and after 1, 3, 6, and, in some cases 9 months of frozen storage duplicate specimens of each variable were prepared for and examined by SEM for texture-related structural damage due to ice crystal formation and growth, employing procedures described in Giddings and Hill (1976). Results are in the form of electron photomicrographs, visual comparison of which reveals differences resulting from the different freezing and frozen storage methods. Specifically, differences in mode of ice crystal formation and growth, and the effects on tissue structural integrity and gross morphology are readily observable.

RESULTS AND DISCUSSION

Freezing rate data were in general agreement with the freezing time monograph (freezing time in minutes versus thickness in inches) of Bucholz and Pigott (1972) for unpackaged low-fat fish muscle immersed in liquid "Freon" Food Freezant, and the known properties of the two liquid freezants. That is, under the conditions employed the average center temperature of the shrimp and crabs monitored in "Freon" dropped

steadily from 0 °C without an initial lag (reflecting the considerable surface heat transfer coefficient of "Freon"), reaching −26.5 °C in 2 min and in 5 min respectively, reflecting the differing muscle thicknesses of the two species and a possible heat transfer-retarding effect of the crab core shell. As the internal temperature approached freezant temperature (ca −30 °C) the rates diminished as expected, and final temperature was approached more-or-less asymptotically (i.e. between about −26.5° and −30 °C). In the case of LN_2 immersion the average rate plots exhibited the expected initial lag or shoulder between 0 and approximately −10 °C (which includes the so-called critical zone of maximum water-ice transition), reflecting the known surface insulating effect of the generated N_2 gas. Once through this zone average center temperature dropped rapidly ("straight line" portions of the rate plots), reaching −190 °C from 0 °C in about 36 s for the shrimp and in about 1¼ min for the crab cores. As was the case with "Freon," the temperature of the LN_2 was approached more-or-less asymptotically between about −190 °C and the ultimate −196 °C. In both liquid freezants the observed variation in rates due to variation in muscle thickness within each species diminished as center temperature approached freezant temperature, the variation being greatest at temperature about midway between 0 °C and freezant temperature. Further, it was estimated that a typical unpackaged crab core warms from LN_2 temperature to around −30 °C in a typical commercial walk-in frozen storage room in on the order of an hour when left exposed in the center of the room. This estimate is based upon a few trials employing individual crab cores with recording thermocouple remaining inserted during warming in a −14 °C freezer storage room immediately following freezing. Individual shrimp would, of course, warm from LN_2 temperature to conventional freezer storage temperature in less time than the crab cores. Individual or bulk packaging would be expected to slow the "come-up" rate.

Just as cryogenic freezing can give rise to stress-cracking or shattering of fragile material such as crustacean muscle (Timbers 1974) warming from cryogenic freezant temperature to conventional frozen storage temperature is known to induce growth of some ice crystals at the expense of others via migratory recrystallization, which arises as a result of the vapor pressure differential among ice crystals having different radii of curvature. During preparation of samples for SEM we observed unmistakable evidence of stress cracking of LN_2-frozen crab and shrimp meat. It should be noted, however, that we employed direct immersion into LN_2 whereas industrially it is normally applied as a spray in a tunnel. It is probable that the latter mode of application avoids stress cracking by slowing the freezing rate somewhat. For example, Fennema (1975)

states that shrimp freeze in about 9 min in a commercial liquid nitrogen freezer. In comparison, shrimp were frozen in this work in seconds of immersion in LN_2, and in about 2 min immersion in Freon food freezant. In regard to migratory recrystallization during post-freezing warming, the figures that follow indicate that this was insignificant when compared with crystal growth during frozen storage. Because of its relatively high subfreezing temperature coupled with its high surface heat transfer coefficient "Freon" provides a very high rate of freezing without either the possibility of stress cracking during freezing or the requirement for post-freezing warming. In this context Sheldon (1975) opines that "there is little technological advantage to be gained in freezing in less than 5 min; indeed, some delicate foodstuffs can be irreparably damaged if frozen too quickly, although this may not be apparent until the product reaches the consumer." He went on to state that "not only can cold storage negate much of the advantage of very rapid freezing, but it is a fact that any freezing process is only as good as the thawing it receives later."

It is evident from examining Fig. 1 and 2 that crab and shrimp muscle slowly frozen at and stored at $-29°$ and $-10°C$ exhibits "extracellular" ice crystal formation and growth with associated dehydration and shrinkage/compaction as the extracellular crystals grow at the expense of "intracellular" water, and in doing so dehydrate and compress the tissue somewhat. After 6 months compressed fragments are evident. In contrast, "Freon"-frozen crab (Fig. 3 and 4) and shrimp (Fig. 5), and LN_2-frozen crab (Fig. 6 and 7) and shrimp (Fig. 8) exhibit exclusively intracellular ice crystal formation and growth, the fibers remaining essentially intact and hydrated albeit rather "pocked" with ice cavities after several months of frozen storage. The overall appearance of the "Freon" and LN_2 frozen samples is virtually the same at any given storage time, which is to be expected since both freezing rates are well within the rate limit for exclusively intracellular formation of tiny ice crystals which grow at the expense of one another during frozen storage. It can be clearly seen that with the passage of time fewer, larger intracellular ice cavities are evident. Also, it is evident from the appearance of the "zero time" LN_2-frozen samples that recrystallization during warming to freezer storage temperature has no significant impact on gross structure. The greater amount of drip loss on thawing of slowly frozen tissue compared with that rapidly frozen is no doubt at least in part due to the extracellular accumulation of water in the form of ice crystals growing in the interstices between fibers. With rapidly frozen tissue the water remains essentially within the fibers during freezing/frozen storage, and, therefore upon thawing. Figures 9 and 10 illustrate the point that at zero time immediately after freezing there is very little difference in gross

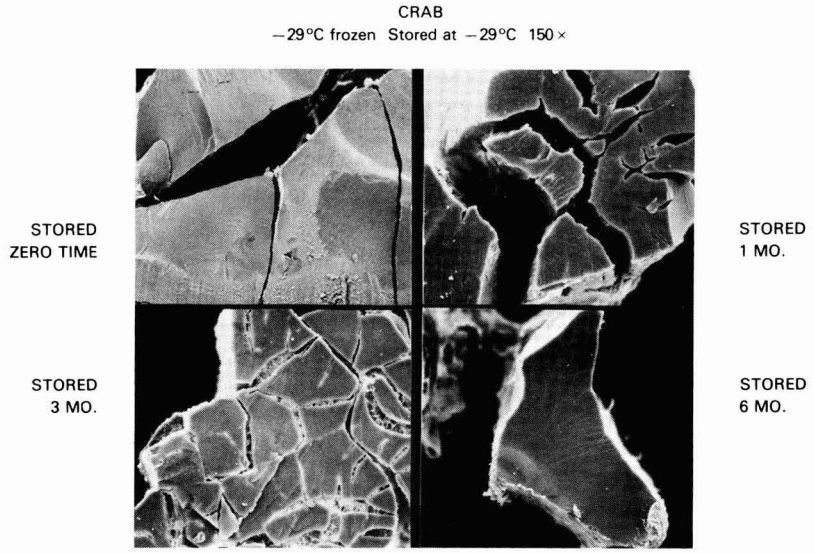

FIG. 1. EFFECT OF STORAGE AT −29°C ON CELLULAR CHANGES OF CRABMEAT FROZEN AT −29°C.

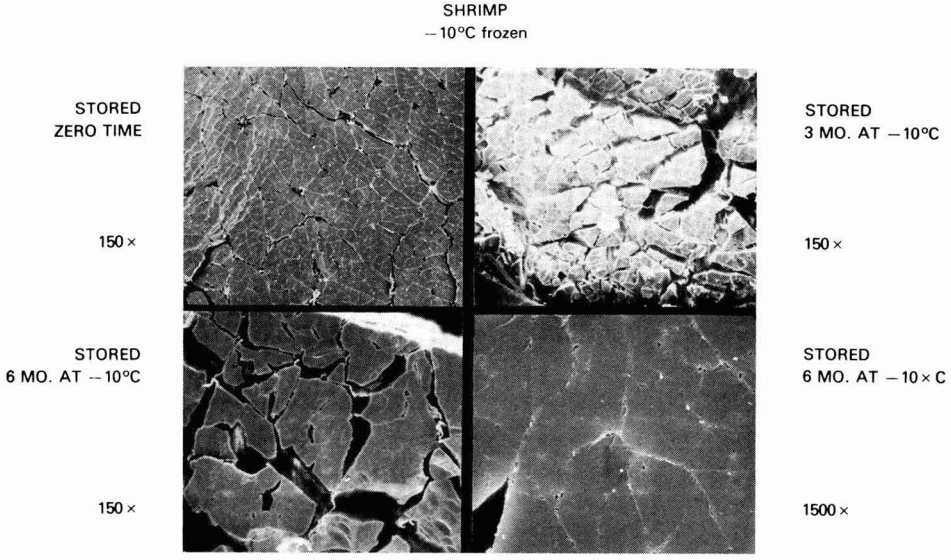

FIG. 2. EFFECT OF STORAGE AT −10°C ON CELLULAR CHANGES OF SHRIMP FROZEN AT −10°C

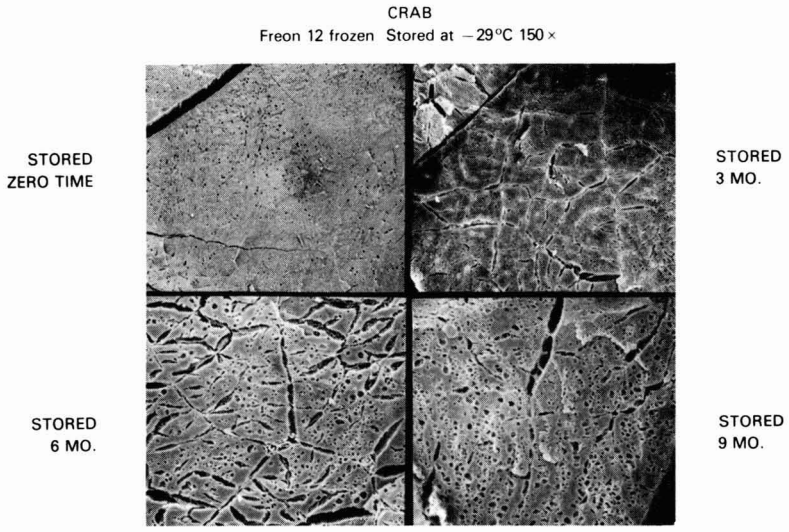

FIG. 3. EFFECT OF STORAGE AT −29°C ON CELLULAR CHANGES OF CRAB FROZEN IN FREON 12 (150 TIMES MAGNIFICATION)

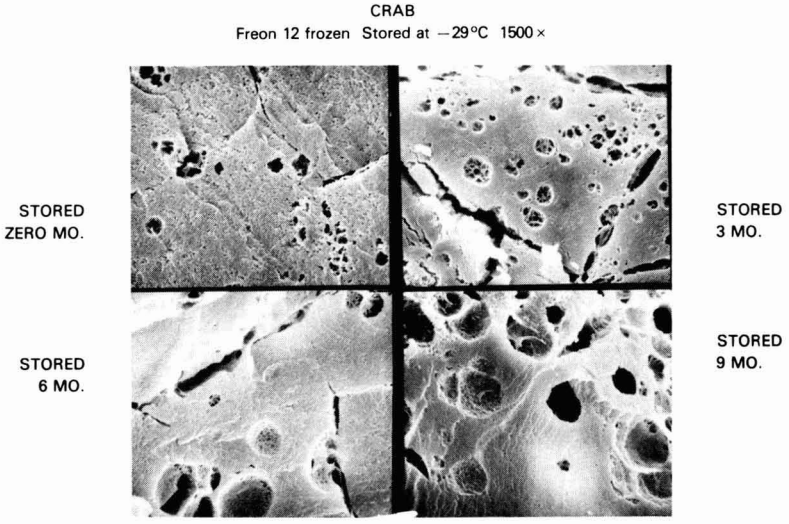

FIG. 4. EFFECT OF STORAGE AT −29°C ON CELLULAR CHANGES OF CRABMEAT FROZEN IN FREON 12 (1500 TIMES MAGNIFICATION)

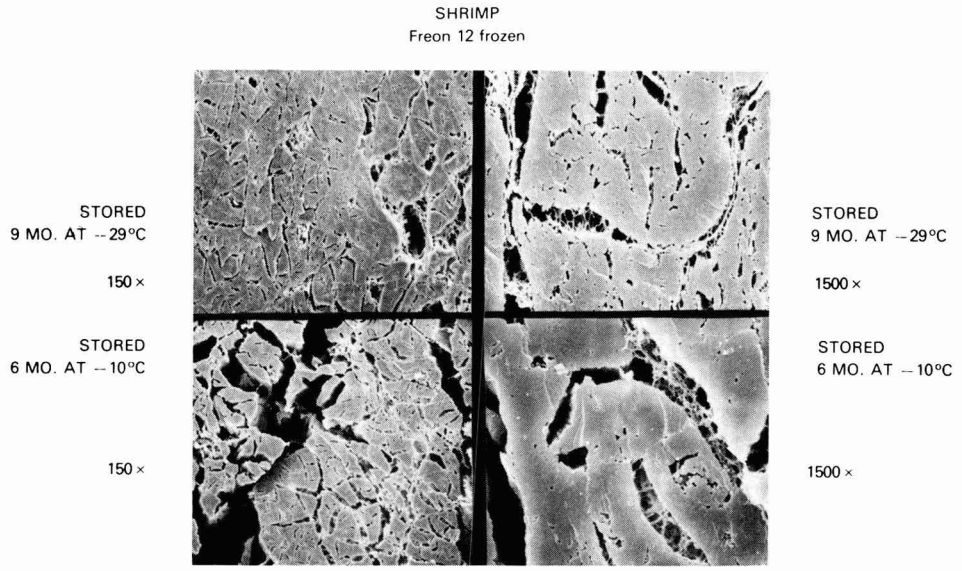

FIG. 5. EFFECT OF STORAGE TEMPERATURE ON CELLULAR CHANGES OF SHRIMP FROZEN IN FREON 12

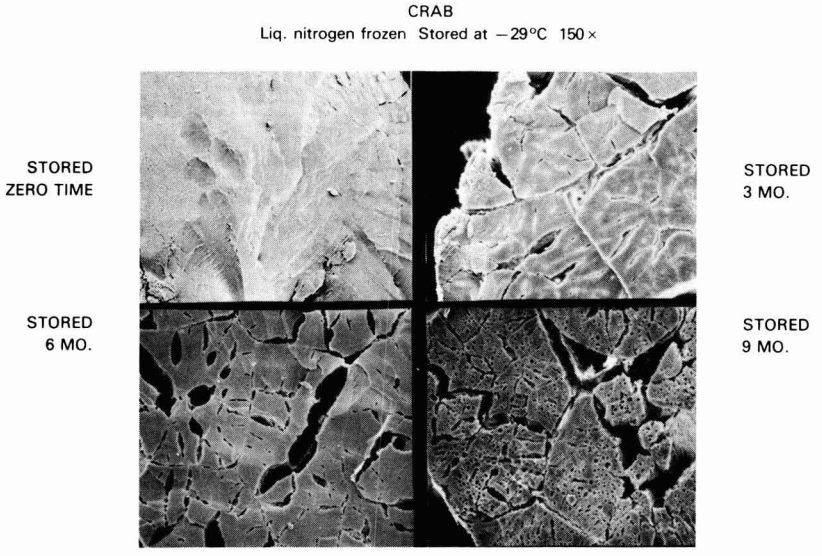

FIG. 6. EFFECT OF STORAGE AT −29°C ON CELLULAR CHANGES OF CRABMEAT FROZEN IN LIQUID NITROGEN

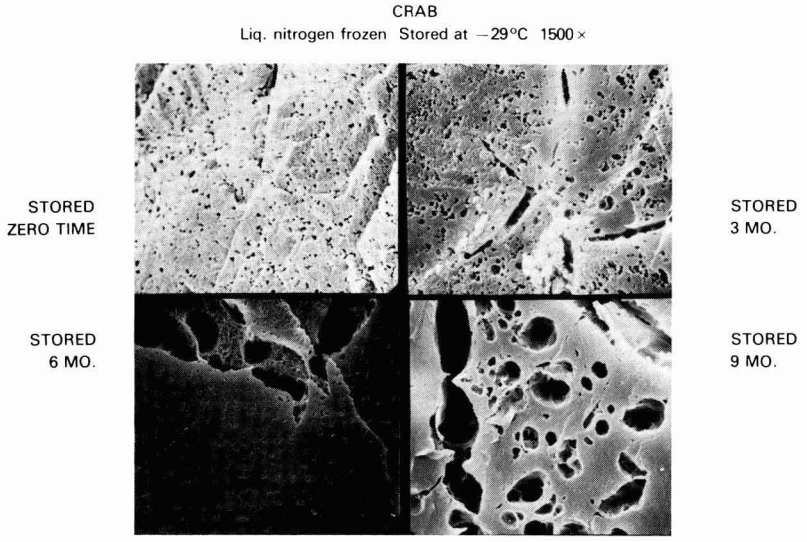

FIG. 7. EFFECT OF STORAGE AT −29°C ON CELLULAR CHANGES OF CRAB FROZEN IN LIQUID NITROGEN (1500 TIMES MAGNIFICATION)

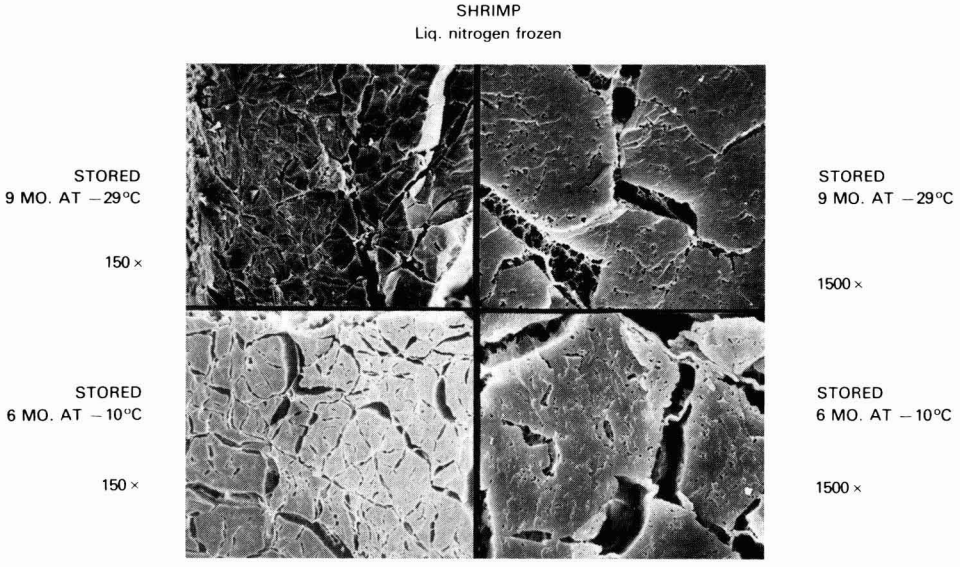

FIG. 8. COMPARISON OF STORAGE AT −10° VERSUS −29°C ON LIQUID NITROGEN FROZEN SHRIMP

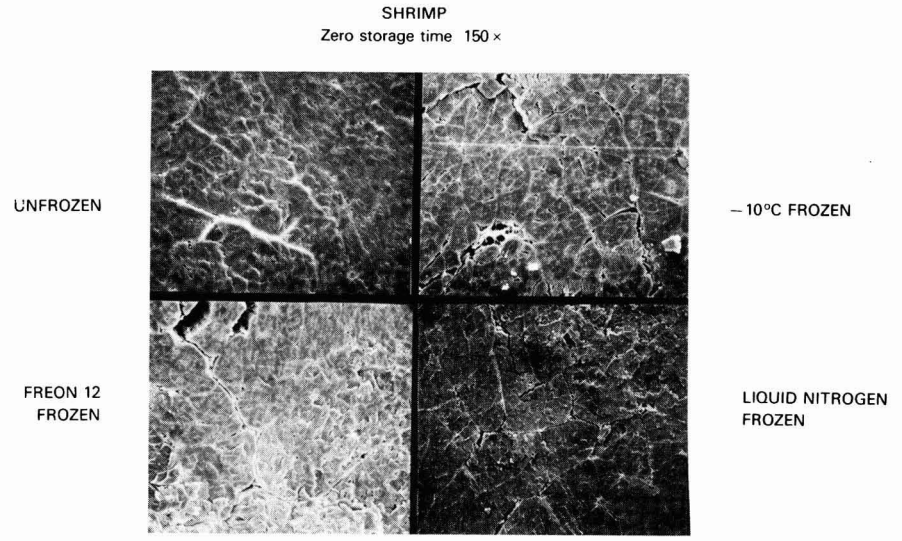

FIG. 9. EFFECT OF FREEZING RATE ON CELLULAR CHANGES IN SHRIMP (150 TIMES MAGNIFICATION)

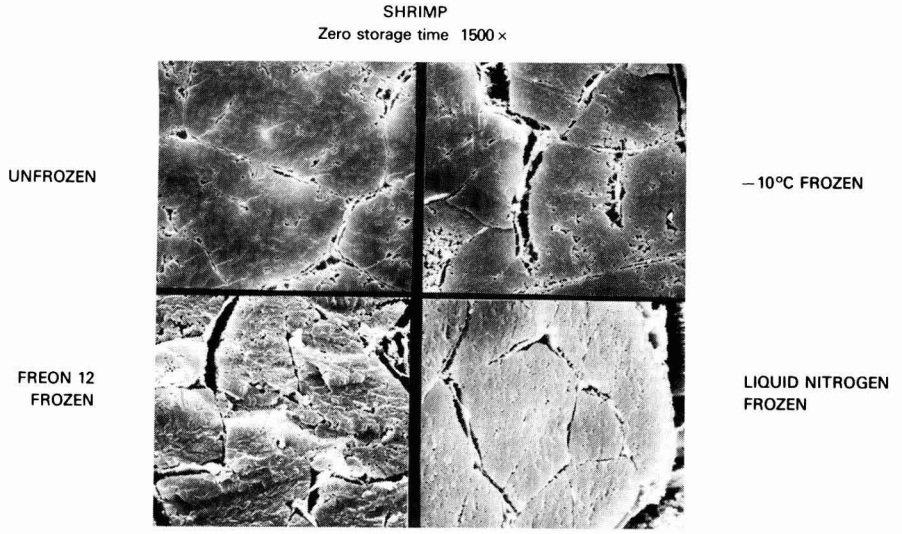

FIG. 10. EFFECT OF FREEZING RATE ON CELLULAR CHANGES IN SHRIMP (1500 TIMES MAGNIFICATION)

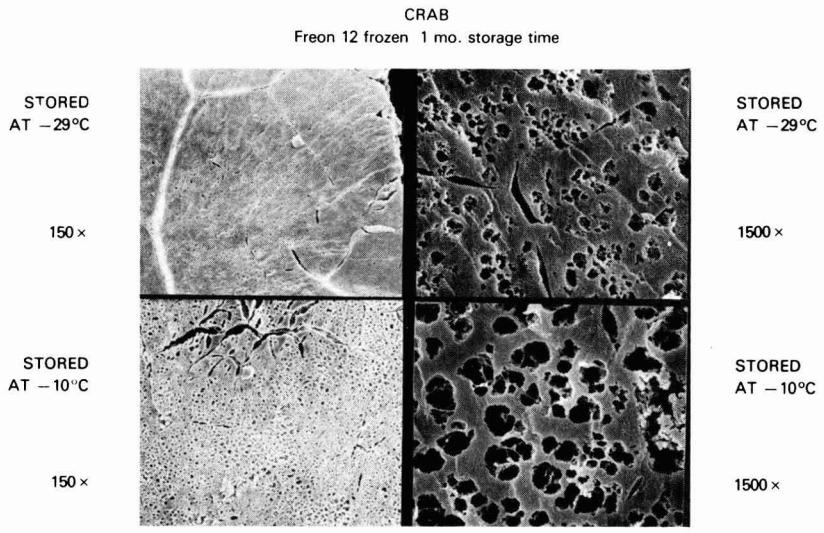

FIG. 11. EFFECT OF STORAGE TEMPERATURE FOR ONE MONTH ON CELLULAR CHANGES IN FREON 12 FROZEN CRABMEAT

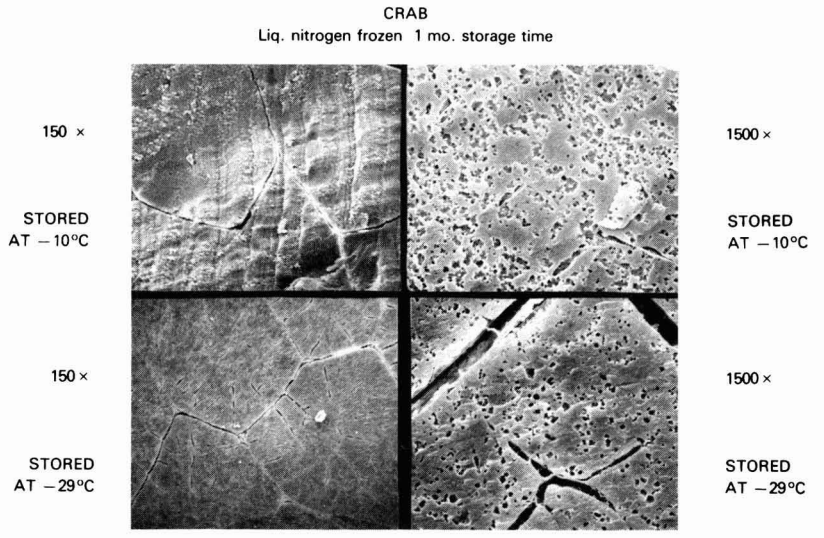

FIG. 12. EFFECT OF STORAGE TEMPERATURE FOR ONE MONTH ON CELLULAR CHANGES IN LIQUID NITROGEN FROZEN CRABMEAT

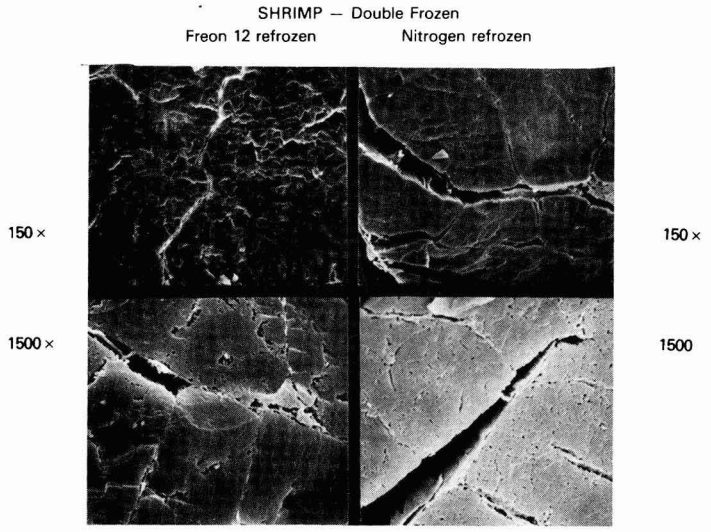

FIG. 13. EFFECT OF FREEZING TWICE ON CELLULAR CHANGES IN SHRIMP

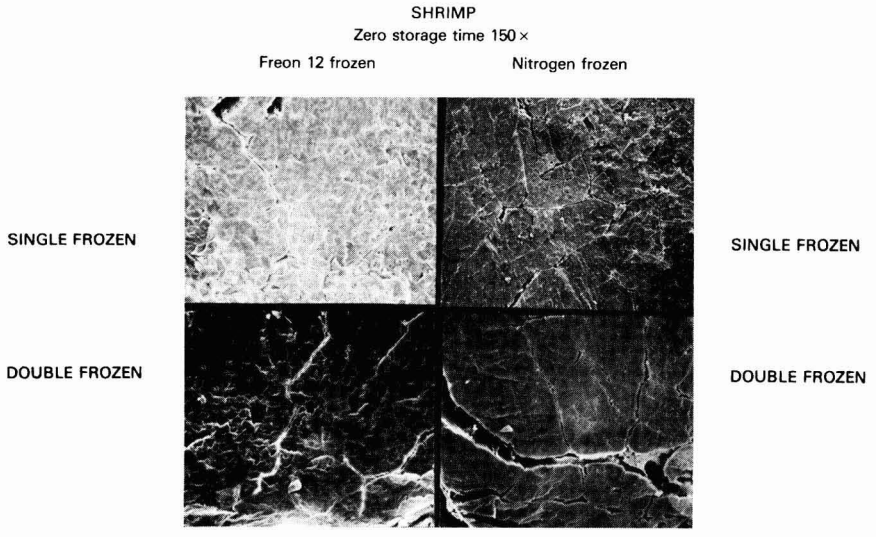

FIG. 14. EFFECT OF FREEZING TWICE BY FREON 12 VERSUS LIQUID NITROGEN ON CELLULAR DAMAGE IN SHRIMP (150 TIMES MAGNIFICATION)

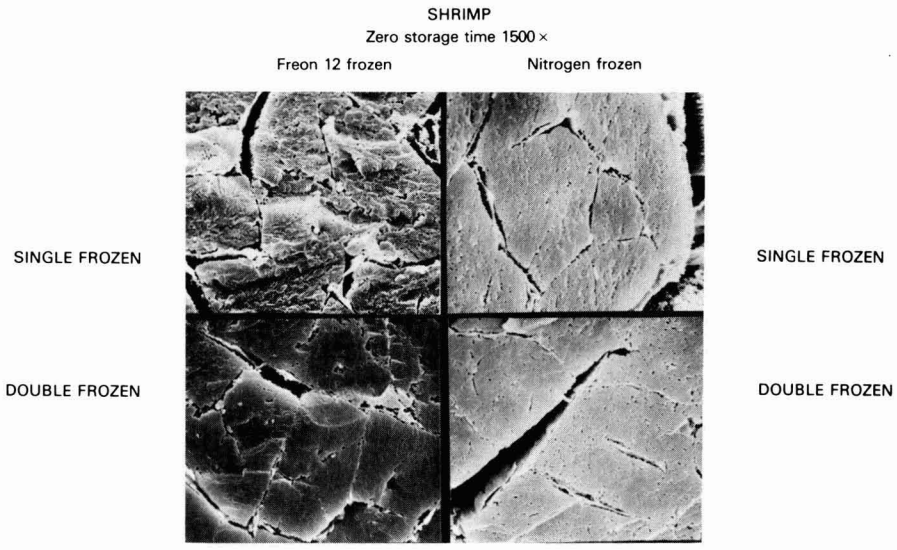

FIG. 15. EFFECT OF FREEZING TWICE BY FREON 12 VERSUS LIQUID NITROGEN CELLULAR DAMAGE IN SHRIMP (1500 TIMES MAGNIFICATION)

appearance when comparing unfrozen tissue and that frozen slowly (−10°C), frozen in "Freon", and frozen in LN_2. This underscores the point that most of the freezing preservation-connected damage develops not during the actual freezing (at the worst one occasionally sees evidence of some interfiber disruption immediately after slowly freezing) but rather, during post-freezing storage and handling.

Figures 11 and 12 and Fig. 5 and 8 illustrate the benefits of frozen storage at −29°C versus −10°C with respect to structural damage due to post-freezing ice crystal growth. Particularly in the case of "Freon"-frozen crab ice crystal growth was noticeably more advanced after 1 month at −10°C than at −29°C. The shrimp micrographs (Fig. 5 and 8) reveal that both "Freon" and LN_2-frozen shrimp held for 9 months at −29°C were, if anything, in a slightly less advanced state of structural deterioration than comparable samples held for 6 months at −10°C. Finally, Fig. 13, 14 and 15 illustrate that within the limits of detection by the SEM method there is no evidence of damage due to double freezing when compared at zero time with once-frozen cooked samples, and that "Freon" and LN_2-refrozen samples are virtually identical in appearance. Earlier work with raw-frozen tissue revealed that if the frozen tissue was thawed in the fixative (i.e. glutaraldehyde solution) ice crystal cavities could be readily seen in the SEM micrographs. If complete thawing was allowed to proceed before fixing, tissue fluid would fill the cavities and

largely obliterate evidence of ice crystal formation and growth. This undoubtedly occurs during thawing of raw-frozen shrimp before commercially cooking and refreezing. Additionally, the cooking process would further erase any signs of ice crystal formation and growth in the raw tissue. Thus it can be concluded from the latter three micrographs that, with respect to gross structural integrity and morphology, double freezing under the conditions employed is no more damaging than cooking and freezing fresh shrimp, and, again, the main damage takes place during frozen storage following the final freezing.

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OSMOTIC CONCENTRATION OF FRUIT SLICES PRIOR TO FREEZE DEHYDRATION[1]

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ABSTRACT

Organoleptic quality of freeze-dried foods can be improved by increasing the solids content of the food material to levels of 25—35%. This also results in a reduction of the water load to the freeze-drier, which greatly improves the economics of the process. For solid foods, such as fruit slices, the increase in solids concentration is achievable by an osmosis process. Sucrose has generally been the solute of choice, but economic considerations are indicating that the suitability of new osmosis solutes should be evaluated.

Several mixed osmosis solutes were evaluated for their effectiveness in concentrating apple slices prior to freeze-drying. Kinetics of water loss and solute uptake were determined for solutions of differing composition and concentration. Several of the osmotically preconcentrated freeze-dried apple slices were evaluated for organoleptic acceptability.

INTRODUCTION

Freeze-drying has long been regarded as a preservation process which yields products of high organoleptic quality. This quality, however, will depend on the process conditions used. For example, it has been shown that the retention of volatile organic compounds (models of flavor components) following freeze-drying of liquid materials depends on the choice of freezing rate and solids concentration of the liquid feed material. Flink (1975a, b) and Thijssen (1975) have reviewed this work. They note that with liquid food systems, retention of flavor compounds was highest with

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slow freezing and when the initial solids concentration was 25—30% or above.

For solid food materials, such as meats, vegetables or fruits, the freezing rate is controllable, but regulation of solids content is difficult. Flink (1975a) reported on the improvement of organoleptic quality of freeze-dried fruit products following an osmotic concentration step, in a 60% sucrose solution, resulting in solids concentrations above 25%.

Ponting *et al.* (1966a,b, 1973), described a process for removal of 50% of the water of fruit pieces by mixing with dry sucrose, or by immersion in concentrated solutions (65—75% solids) of sucrose or invert sugar. They indicated that the final products can be air or vacuum-dried, or dehydrofrozen but did not examine freeze-drying. Farkas and Lazar (1969) conducted further evaluations of the effects of temperature and sucrose concentration on rates of osmotic dehydration. They also report on scale-up to a pilot plant process. Hope and Vitale (1972) described the application of osmotic dehydration for concentration of banana, plaintain and ripe mango using 67% (w/w) sucrose solutions and green mango using 25% (w/w) sodium chloride solution. Pader and Richberg (1968) also describe an osmosis step as a preconcentration prior to air or vacuum-drying. They note that it is necessary to use a crystallizing sugar, and claim an advantage to sulfite treatment instead of blanching for prevention of browning, since structural integrity of the tissue will be retained. On the same basis, freezing of the tissue is undesirable.

Garcia *et al.* (1974), found that pretreatment in 65% sucrose, did not affect quality of dried fruit. Dixon *et al.* (1976), used a combination of osmotic-dehydration and vacuum-drying to produce dry apple slices.

Dymsza (1975) described a process in which solid food products were osmotically dehydrated by immersion in an alkene glycol, such as propylene glycol to produce a shelf-stable product. While there is preservative action associated with the reduction of water content, the infusion of the preservative into the piece is of more significance. Camirand *et al.* 1968) coated food pieces with a thin calcium pectate membrane. The coated pieces were then immersed in a 75° Brix invert sugar: sucrose solution (50:50) for the osmotic dehydration. The presence of the membrane prevented uptake of the solute by the food piece, but allowed removal of water.

The current study investigates the kinetics of osmotic concentration for solute systems of varying composition and concentration. In particular, several mixed solute systems were considered as a means for reducing the requirement for sucrose.

MATERIALS AND METHODS

Materials

Reagent grade sucrose and sodium chloride, and commercial lactose (Purity Cheese Co., Mayville, Wisconsin) and maltodextrin of 15 DE (Maltrin-15, Grain Processing Corp., Muscatine, Iowa) were used to prepare osmosis solutions. In addition, sucrose and lactose were evaluated in the dry state for their water removal capabilities.

McIntosh apples were purchased at a local supermarket. At those times of the year when apples were available only from controlled atmosphere storage, care was taken to avoid selecting individual apples with obvious softening of the tissue. Studies were suspended during the summer when apple quality was relatively poor.

Preparation Methods

The osmosis solutions were prepared as pure solute or mixed solute systems with total concentrations generally being 25—60% (w/w). Sodium chloride was examined at concentrations of 5—25% (w/w). All osmosis solutions contained (in addition to the osmotic agents) 0.52% ascorbic acid and 0.14% malic acid to prevent browning of the apple slices during handling and subsequent processing.

Studies on the osmotic preconcentration of fruit slices have been conducted by a variety of methods, including:

(1) Agitating fruit in a "static" solution
(2) Static contacting of fruit and solution
(3) Contacting of fruit and solution while the fruit is under vacuum
(4) Mixing of fruit pieces with dry sugar
(5) Flow of solution through a static bed of fruit

The work reported here will concentrate on the first method, agitating the fruit in a "static" solution. For each experiment, 400 g of osmosis solution of known concentration are placed in a one-liter graduated cylinder with a motor driven porous plunger which gently agitates the fruit pieces in the osmosis solution by a reciprocating motion. Apples were manually peeled, quartered and the quarters cut into uniform slices of 3-4 mm thickness. Each apple slice was individually weighed and coded by wrapping with a few turns of colored thread. To the 400 g of solution were added 5 slices totaling about 20 g of wet weight. An excess of osmosis solution was used to limit concentration changes due to uptake of water from the apple slices and loss of solute to the slices. A slice was

removed from the osmosis solution at 0, 0.5, 1,2,3, and 4 h for gravimetric determination of the water loss and solids uptake. Refractometric measurements are made on the osmosis solution at these times to monitor changes in concentration.

The water loss and solids uptake can be determined by gravimetric measurement alone if it is assumed that under the conditions used, the solutes present initially in the apple slice will not diffuse against the total solids concentration gradient into the concentrated osmosis solution. The total wet weight (tw) of the apple slice is determined upon removal from the solution and the total solids weight (ws) determined by first freeze-drying followed by drying in a vacuum oven at 50 °C for 24 h. Now, with the assumption given above, and using the coding system which allows knowledge of the initial slice solids (wso) and water (wwo) contents, the solids gain can be defined as:

$$SG = \frac{(ws - wso)}{(wso + wwo)} \times 100[\text{g solids/100 g initial wet apple}] \quad (1)$$

and the water loss as:

$$WL = \frac{(wwo) - (tw - ws)}{(wso + wwo)} \times 100\ [\text{g water/100 g initial wet apple}] \quad (2)$$

The total solids at any time is defined as:

$$TS = \frac{ws}{tw} \times 100\ [\text{g solids/100 g apple}] \quad (3)$$

In a few cases, a different method for conducting the initial fruit-solution contact was investigated. The apple slices with coded threads are put into a 4-l vacuum chamber, which is evacuated with a mechanical pump to an absolute pressure below 10 torr. While the system remains under vacuum, osmosis solution is allowed to enter the chamber until the apple slices are completely immersed; an excess of solution is used. The system remains under vacuum throughout the length of the experiment, except when samples are removed for analysis. Water loss and solids uptake are measured gravimetrically as described above.

The suitability of dry sugars for osmotic concentration of apple slices was also considered. Fruit slices were mixed with an equal weight of dry

lactose, sucrose or lactose/sucrose mixtures. They were agitated periodically by shaking over the 24 h holding period, the apple slices were rinsed quickly (2—3 s) to remove adhering sugar prior to determining water loss and solids uptake. Since kinetics were not being evaluated, color coding was not necessary.

All the osmotic concentration studies were conducted at room temperature (approximately 23 °C).

Selected osmosis conditions were chosen for preparation of larger amounts of concentrated apple slices for subsequent freeze-drying. The apples were placed on trays and frozen at −20 °C. They were hard frozen in liquid nitrogen prior to insertion in the Virtis freeze-drier (Model 10-MRTR). Drying was conducted at a pressure below 100 mTorr and with the heating plates at ambient temperature. With the product loading used, the drying time was approximately 48 h.

Organoleptic evaluations of the dried products were conducted using a nine point hedonic rating difference test with a minimum of 12 trained panelists. Products were tested for quality of taste and texture. The results were analyzed statistically according to Larmond (1970).

Osmosis Kinetics Analysis

Mass transport data of the type given in Equations (1—3) can be analyzed according to standard techniques used for obtaining diffusion coefficients, assuming unsteady state Fickian diffusion. By means of simplifying assumptions to the indefinite series expressions, "diffusion coefficients" can be determined by plotting:

$$C \text{ versus } (\text{time})^{1/2} \tag{4}$$

and measuring the slope of the resulting straight lines.

For the unsteady state Fickian diffusion model to exactly apply it is necessary that external solution concentrations remain constant, and that resistance at the surface is negligible compared to internal diffusion resistance. In this analysis, it is assumed that solution conditions remain essentially constant since there is a large volume of osmosis solution relative to the amount of apple slices.

The condition of total mass transfer resistance being internal to the piece is not met in all cases, especially at higher concentrations of osmosis solution. Thus, the transport coefficients obtained are overall mass transport coefficients or pseudo "diffusion coefficients."

Several different parameters can be used as the concentration term of equation (4). Four choices were considered. Water lost per 100 g of initial apple sample neglects the effect of solute uptake and does not account for

differences of initial water content of the various apple samples. This presentation presumably is related to water diffusivity, but the changing apple solids content, due to uptake of solute will alter the driving force for water flow beyond that due to the diffusive loss of water alone. Presenting the fraction of initial water which has been lost accounts for differences of initial water contents of the apple samples, but still does not account for changes in solids content due to solute uptake. Using the change in weight percent total solids does not separate the solute uptake from the water loss, but it does measure a parameter of importance in this study, the increase of solids content prior to freeze drying. An improvement is presentation of the percent total solids change on a unit initial total solids basis (i.e. normalized solids content, NSC), so that variations between initial samples can be considered, and this was the method of presentation chosen:

$$(NSC) = K(t)^{1/2} \tag{5}$$

where K is defined as the mass transfer coefficient.

The mass transport coefficient (K) is determined from the slope of the NSC versus $(t)^{1/2}$ curves. However, it can be recognized that in practice there is a rapid uptake of solute within the first one-half hour of osmosis which results in a displacement of the effective "initial" solids content for the remainder of the osmosis period. It is possible to define a K value for the overall osmosis process (total solids gained plus water loss) by including all the data points (i.e. including time = 0) in the determination of the slope of the NSC versus $(t)^{1/2}$ curve. If, however, the zero time data point is omitted, then the "K" value (called K') reflects primarily the water loss, with solids gain subsequent to the rapid initial uptake having a much smaller effect.

The gravimetric data for osmosis of apple slices with 50% sucrose solution (Table 1) is illustrated in Fig. 1. The plots of NSC versus $(t)^{1/2}$ for the 50% sucrose solution are shown in Fig. 2. In drawing the regression lines, the zero time point was included in one case and omitted in the other. As noted above, the lines represent the overall K value and the K' value associated primarily with water loss. For the case of the water loss K' value, the higher intercept value reflects the effective NSC value at zero time (i.e. increase in solids due to the rapid uptake of solute).

In this report, K values will be reported since they more closely reflect the interest in change of total solids to a level desired for freeze-drying.

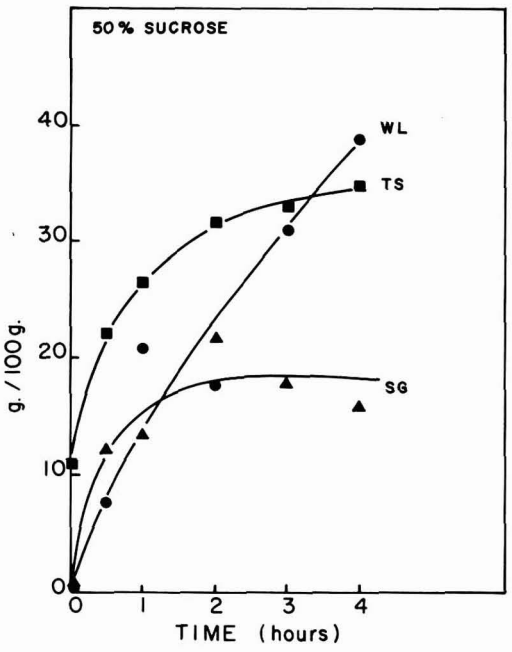

FIG. 1. MASS TRANSPORT DATA FOR OSMOTIC CONCENTRATION OF APPLE SLICES WITH 50% SUCROSE SOLUTION

SG = solids gained (g solids/100 g initial wet apple)
WL = water loss (g water/100 g initial wet apple)
TS = total solids (g solids/100 g apple tissue at time of measurement)

RESULTS AND DISCUSSION

Effect of Solute Concentration

The overall mass transport data for osmosis with sucrose are given in Table 1. The normalized solids content are shown in Fig. 3a and 3b as a function of time and $(\text{time})^{1/2}$, respectively. The K values, obtained from the slopes of the lines in Fig. 3b, are tabulated (Table 2) with the K' values and their corresponding extrapolated NSC values at zero time (intercept). Also given in Table 2 are the gain of solids content after one-half hour of osmosis (i.e., a measurement of the rapid uptake) and the average gain of solids at the end of the osmosis process (average value for 2, 3, and 4 h). The intercept is a measure of the rapid solids uptake, since in most cases little additional solid is gained after the first hour of immersion (Table 1, Fig. 1). K values increase with sucrose concentration; K' values also increase, though 50 and 60% values are about the same. Since

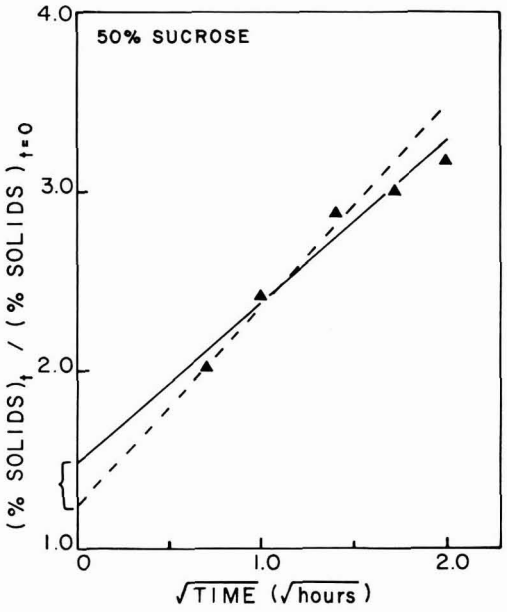

FIG. 2. NORMALIZED SOLIDS CONTENT FOR APPLE SLICES VERSUS (TIME)$^{1/2}$ WHEN CONCENTRATED IN A 50% SUCROSE SOLUTION, SHOWING METHOD FOR DETERMINING K AND K' VALUES

(K — dashed line; K' — solid line)

most of the solids uptake occurs within the first one-half hour, this would indicate that the rates of water removal are similar for the 50 and 60% sucrose concentrations after this initial solute uptake, which is much greater for the 60% than 50% sucrose concentration. This reflects the increase in intercept value for the K' evaluations.

The K values and average total solids content at the end of the osmosis period for all systems examined are given in Table 3. The effect of concentration on K value and final apple slice solids content is similar to that noted above for the series of sucrose solutions.

Effect of Agitation During Osmosis

In a set of experiments with a different batch of apples (hence slightly different K values), the influence of agitation on the mass transport coefficients was investigated. Figure 4 shows that the gentle agitation used in this study has little effect on osmosis rate and K values at low osmosis

Table 1. Mass transport data for osmotic concentrations of apple slices

Osmosis Solute Concentration	Time (h) 0	½	1	2	3	4
25% Sucrose						
SG[1]	0	7.6	7.4	7.2	7.7	8.5
WL[1]	0	−7.0	−1.8	−3.5	−0.29	+5.1
TS	12.4	17.4	17.9	17.8	18.6	20.1
NSC	1.00	1.40	1.44	1.44	1.50	1.62
40% Sucrose						
SG	0	10.6	12.6	15.4	12.0	13.2
WL	0	2.9	8.0	16.3	22.1	25.0
TS	12.5	21.4	24.0	28.1	27.4	29.1
NSC	1.00	1.71	1.92	2.25	2.19	2.33
50% Sucrose						
SG	0	12.3	13.5	22.0	17.9	15.9
WL	0	7.7	20.9	17.8	31.0	38.9
TS	11.0	22.2	26.6	31.8	33.1	35.0
NSC	1.00	2.02	2.42	2.89	3.01	3.18
60% Sucrose						
SG	0	18.5	19.7	19.5	22.5	25.5
WL	0	22.0	32.0	27.2	43.3	48.7
TS	10.8	30.4	34.7	32.7	42.2	36.5
NSC	1.00	2.81	3.21	3.03	3.91	3.38

SG = Solids gain (g solid/100 g initial apple)
WL = Water loss (g water/100 g initial apple)
TS = Total solids (g solids/100 g apple)
NSC = Normalized solids content = $(TS)_t/(TS)_0$

Table 2. Mass transport coefficients (K, K') and solids gain for sucrose osmosis solutions

Sucrose Concentration	K[1] ($h^{-1/2}$)	r^2	K'[3] ($h^{-1/2}$)	r	Intercept	Average Solids Gain Initial[4]	Final[5]
25	0.27	0.85	0.15	0.80	1.28	7.6	7.8
40	0.65	0.92	0.46	0.88	1.45	10.6	13.5
50	1.09	0.96	0.88	0.96	1.49	12.3	18.6
60	1.20	0.77	0.87	0.68	2.19	18.5	22.5

[1]Mass transport coefficient (evaluated with zero time data)
[2]Regression coefficient
[3]Mass transport coefficient (evaluated without zero time data)
[4]Increase in solids at ½ h
[5]Increase in solids averaged over 2—4 h

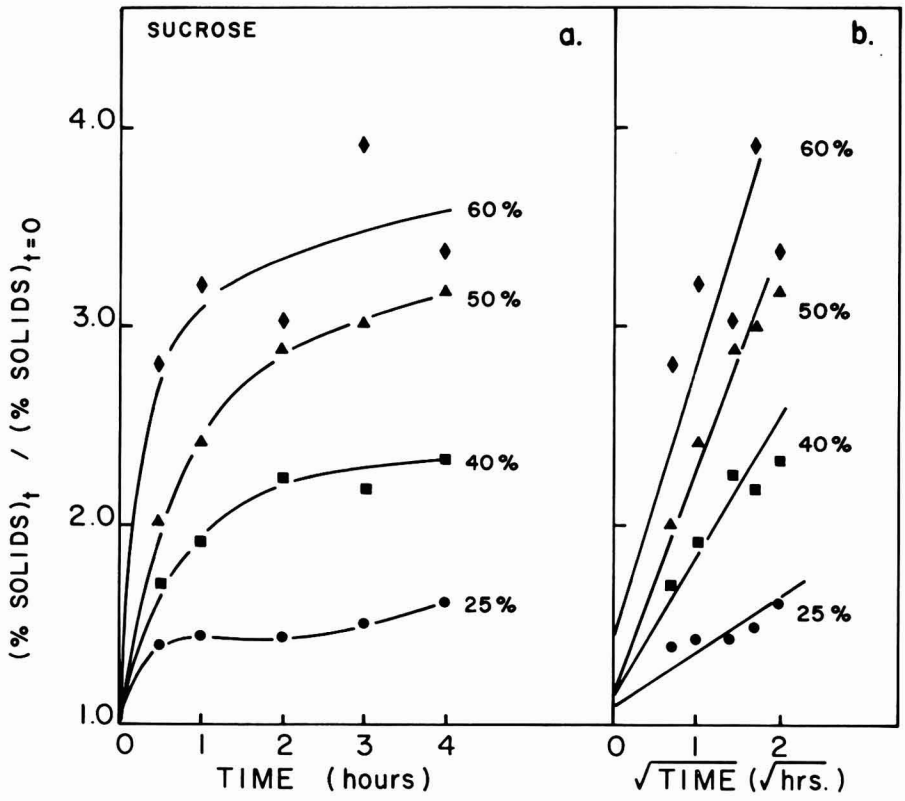

FIG. 3. NORMALIZED SOLIDS CONTENT FOR OSMOTIC CONCENTRATION OF APPLE SLICES IN SUCROSE SOLUTIONS

solute concentrations. As solution concentration increases, however, and there is a concomitant rise in viscosity and resistance to mass transfer, agitation results in higher K values for agitated systems as compared to the non-agitated system.

Solute Uptake Behavior

During the course of osmosis, the apple slices pick up solute. It has been shown that the solids are gained very early in the process and then increase only very slowly during the remainder of the process (Fig. 1; Table 1, 2). While no attempt has been made to measure the spatial distribution of this solute, it is likely to be either located as a thin surface layer, or perhaps in intercellular spaces of the fruit slice.

Table 3 shows the net solute uptake at the latter stages of the osmosis

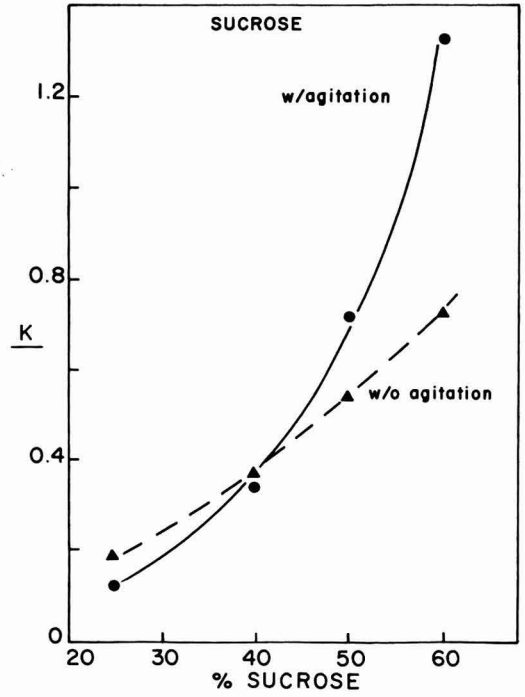

FIG. 4. EFFECT OF AGITATION OF APPLES ON MASS TRANSPORT COEFFICIENTS

process. It can be seen that for the sucrose solutions, the solute uptake increases as the osmosis solution concentration increases. It was noted that samples prepared with agitation have higher levels of uptake than non-agitated samples and that the uptake values of the mixed solutes for solutions of the same total solids content reflect to some extent the uptake values of the individual components at the concentrations present in the mixed solution.

Effect of Solutes

The mass transport coefficients (K) and the average total solids content (averaged over 3—4 h) of the apples for the various osmosis solutions are given in Table 3. The dependence of K values and final piece solids content on total solids concentration for a series of osmosis solutions are shown in Fig. 5 and 6, respectively.

Lactose has a much lower level of sweetness than sucrose and may also become available in increasing quantities as cheese wheys are recovered

Table 3. Mass transport coefficients for osmotic preconcentration of apple slices based on normalized solids content

Total Concentration of Osmosis Solution	Mass Transport Coefficient (K) ($h^{-1/2}$)	Total Solids in Apple Slice[1] (%)
5%		
NaCl	0.11	13.1
10%		
NaCl	0.32	17.7
25%		
Sucrose	0.27	19.4
Lactose	0.19	18.9
Maltodextrin	0.17	15.8
NaCl	0.85	32.3
40%		
Sucrose	0.65	28.3
25% Lactose/15% Sucrose	0.57	29.5
Maltodextrin	0.46	21.5
25% Maltodextrin/15% Sucrose	0.30	22.3
50%		
Sucrose	1.09	34.1
25% Lactose/25% Sucrose	0.81	35.0
Maltodextrin	0.75	29.9
35% Maltodextrin/15% Sucrose	0.67	29.0
15% NaCl/35% Sucrose	1.10	43.5
10% NaCl/40% Sucrose	1.50	49.6
60%		
Sucrose	1.20	39.4
25% Lactose/35% Sucrose	1.05	43.6
45% Maltodextrin/15% Sucrose	1.00	40.3

[1]Averaged for 3- and 4-h periods

and fractionated to recover proteins, leaving a lactose rich fraction. One potential problem is the low solubility of lactose in aqueous solution. The solubility limit for lactose is generally reported to be about 17—20 g of lactose per 100 g of solution. In this study, lactose solutions were prepared at concentrations of 25—28% by first heating the solution to dissolve the solids and then allowing the solution to cool to room temperature before use. This undoubtedly resulted in a supersaturated solution, though in all the studies conducted with pure lactose solutions, no nucleation of crystals was observed during the time period of osmotic treatment. Only once in a 25% lactose/35% sucrose mixed system, very small lactose crystals were observed to form after an extended period of standing.

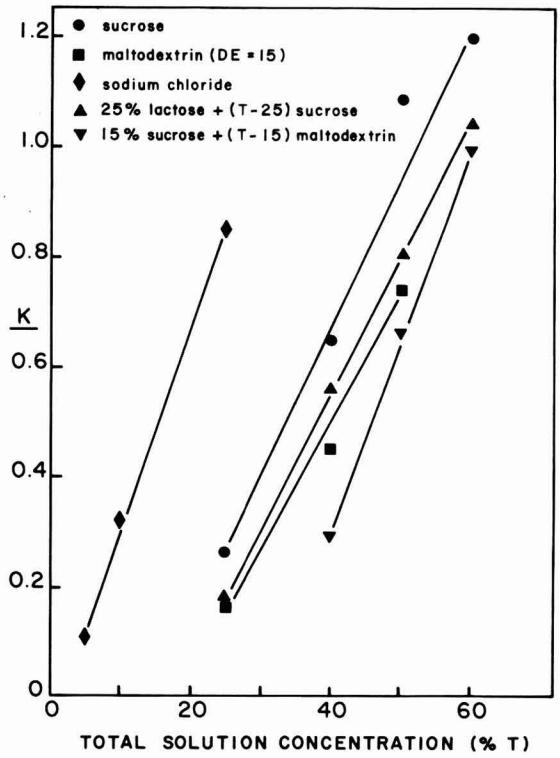

FIG. 5. DEPENDENCE OF MASS TRANSPORT COEFFICIENTS ON SOLUTION TOTAL SOLIDS CONCENTRATION

Osmotic preconcentrations of peach slices and banana slices were attempted using lactose solutions at 20 and 28% solids, while a solution at 25% solids was used with apple slices. A pure lactose solution was not as promising as an osmosis solute, since only slight preconcentration could be achieved.

Mixed Lactose/Sucrose Solutions. The K values for lactose/sucrose mixtures are given in Table 3. It can be seen (Fig. 5) that the K values of the 25% lactose/sucrose mixtures increase with increase in total solids from 40 to 60%.

Lactose solutions near or slightly above their solubility limit in combinations with sucrose at total concentrations of 40, 50 or 60%, give sizeable increases in solids content of the apple slices, which are larger than the sum of the increases which would result from each component at the concentration which is present in the mixture.

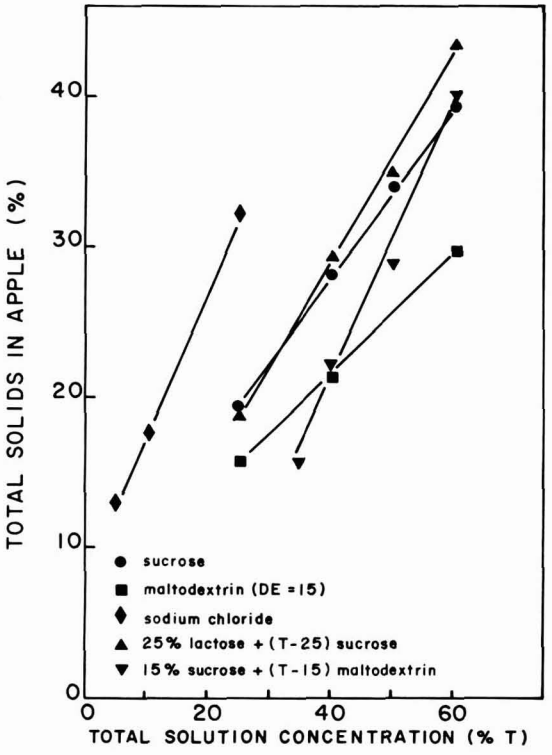

FIG. 6. DEPENDENCE OF FINAL SOLIDS CONTENTS OF APPLE SLICES ON OSMOSIS SOLUTION, TOTAL SOLIDS CONCENTRATION

Sodium chloride has been tested alone at 5—25% solids and in combination with sucrose at a total concentration of 50% solids. The results show that salt-based solutions are very effective for concentrating apple slices. However, the extent to which NaCl can be used as a substitute for sucrose is probably limited due to its saltines. Organoleptic tests indicate that even two-step processes (salt, then sugar) will not be successful.

Maltodextrin was evaluated as an osmosis solute, alone at concentrations of 25, 40, and 50% and in combination with sucrose at total solids concentrations of 40% (25% maltodextrin/15% sucrose), 50% (35% maltodextrin/15% sucrose) and 60% (45% maltodextrin/15% sucrose). The measured K values and final apple total solids contents are given in Table 3. It can be seen that maltodextrin can be used as an osmosis solute at higher total solids concentration, or in mixed systems. The 25% maltodextrin is relatively ineffective.

Effect of Total Solids Concentration in Solution

Table 3 and Fig. 5 and 6 show the effect of total solids content of the osmosis solution on the mass transport coefficients (K) and the final total solids in the osmosed apple slices after 4 h. At the 25% total solids level salt is, as expected, by far the best osmosis solute. Sucrose, lactose and maltodextrin solutions at 25% total solids show similar K values and give apple slices of comparable, but low, final solids content.

At 40% total solids in the solution, the final solids content of the apple slices fall within the range 22—28% solids for all solutes tested. Carbohydrate solutions at 50% total solids have similar K values, though sucrose shows a slight disadvantage. These solutions give apple slices with total solids between 29—35% (which is at a desirable level for freeze-drying).

All solutions at 60% solids are effective, having similar K values and giving comparably high levels of total solids in the apple slices. Sucrose again seems to show a slightly higher rate of osmosis, however.

Osmosis with Dry Sugars

Fruit slices were held (with periodic shaking) for 23 h in dry lactose or sucrose. The results show that while lactose powder is only slightly more effective than saturated lactose solutions in removing water, sucrose is very effective, ending up as a subsaturated solution. With dry lactose a caked region forms, giving a shell of low water permeability around the fruit slice. It appears that this shell prevents further transport of water from the fruit slice.

The osmosis was conducted also with mixed sucrose/lactose systems. The results show (Fig. 7) that while there is a decrease in water removal as lactose replaces sucrose (at constant total amount solids), the mixture acts synergistically. The solids content which earlier studies have shown to be desirable for attaining improved freeze-dried quality can be achieved with a dry 50:50 mixture of lactose and sucrose. The presence of the sucrose apparently gives flow paths for water removal from the fruit piece so that all the lactose is available as a moisture sink.

The apple slices preconcentrated with dry sucrose increased from 11 to 36% solids. However, either the loss of water or the rate of total loss was so great with pure dry sucrose that the slices were highly shrunken, giving a poor appearance. This is in contrast to the good appearance which is obtained when the osmotic pre-treatment is achieved using a 60% sucrose solution. Three hours in a 60% sucrose solution gives apple slices of approximately 30% solids. With the mixed dry solids systems, all samples had reasonably good appearance, being only slightly shrunken.

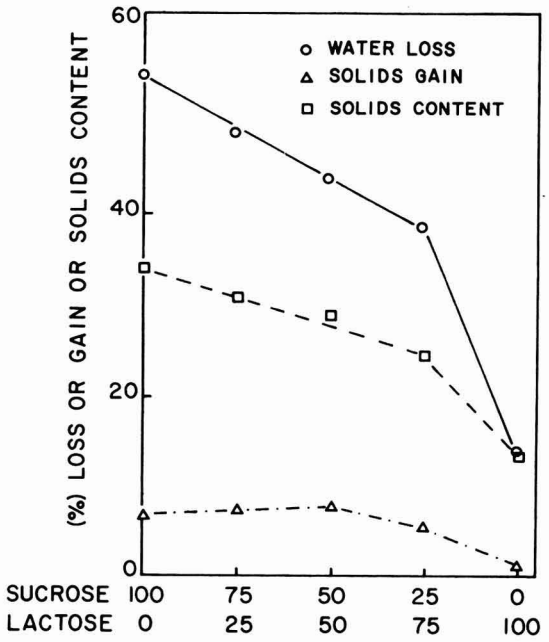

FIG. 7. MASS TRANSPORT DATA FOR OSMOTIC CONCENTRATION IN DRY SUGAR SYSTEMS

(sugar/apples = 1:1 by weight)

Effect of Vacuum Contacting of Fruit and Solution

Evacuation of the chamber containing the apple slices and contacting with solution while under vacuum gave increased uptake of solids in the initial period of contact as compared to the agitated systems of equal composition (Table 4). It is seen that when osmosis was conducted under vacuum a period of 3 h was more than adequate to attain the desired total solids levels, though this was achieved with more solids uptake and less water removal.

Organoleptic Evaluations

Selected, osmotically concentrated freeze-dried apple slices were evaluated in the dry state and following rehydration. Table 5 gives the taste and texture scores on a nine point scale (9 = like extremely; 1 = dislike extremely) for 4 tests on groups of the dried apple slices, and taste scores for rehydrated ground apple, which were generally slightly higher for the mixed osmosis solutes. (The texture of rehydrated samples was not evaluated).

Table 4. Mass transport data for vacuum contacting of apple slices and osmosis solution

Osmosis Solution	Time of Osmosis (h) 0	OV[1]	½	1	2	3	4
Vacuum 60% Sucrose							
SG[2]	0	11.9	28.4	24.6	23.9	35.9	———
WL	0	0	8.6	10.9	22.5	35.9	———
TS	9.9	19.6	32.0	30.5	33.5	45.7	———
NSC	1.00	1.98	3.23	3.08	3.39	4.62	———
60% Sucrose							
SG	0	———	13.5	17.8	16.5	16.7	22.0
WL	0	———	8.5	20.4	35.4	57.0	42.5
TS	11.8	———	24.1	30.4	34.8	48.0	42.5
NSC	1.00	———	2.04	2.58	2.95	4.08	3.60
Vacuum 35% Sucrose							
25% Maltodextrin							
SG	0	19.2	18.4	21.2	19.4	25.1	———
WL	0	- 6.4	2.7	10.3	19.6	20.9	———
TS	12.4	25.2	26.5	30.2	31.9	35.8	———
NSC	1.00	2.03	2.14	2.44	2.57	2.89	———
35% Sucrose							
25% Maltodextrin							
SG	0	———	12.6	12.0	15.1	14.0	10.9
WL	0	———	16.8	14.8	22.3	31.9	34.3
TS	11.5	———	25.3	24.2	28.6	31.0	29.3
NSC	1.00	———	2.20	2.10	2.49	2.70	2.55

[1]OV sample analyzed immediately after contact with osmotic solution

[2]SG = Solids Gain; WL = Water Loss; TS = Total Solids; NSC = Normalized Solids Content (see text)

Table 5. Organoleptic scores for osmotic preconcentrated freeze-dried apple slices

Test Number	Osmosis Treatment	Taste Score (DRY)[1]	Sig.[2]	Texture Score (DRY)[1]	Taste Scores (Rehydrated)[1]	Sig.[2]
1	60% Sucrose	7.50	A	7.00	6.45	A
	40% Sucrose	7.58	A	6.75	7.18	A
	15% Sucrose/25% Lactose	6.83	A	6.33	6.64	A
	15% Sucrose/25% Maltodextrin	6.67	A	6.50	6.00	A
2	15% Sucrose/25% Lactose	7.42	A	7.33	7.70	A
	25% Sucrose/25% Lactose	7.17	A	7.17	7.40	A
	35% Sucrose/25% Lactose	7.50	A	7.25	7.90	A
3	15% Sucrose/25% Maltodextrin	6.92	A	6.82	7.17	A
	25% Sucrose/25% Maltodextrin	7.17	A	7.36	7.58	A
	35% Sucrose/25% Maltodextrin	6.58	A	7.36	7.67	A
4	Two Step: NaCl/Sucrose[3]	3.82	A	6.27	---	---
	10% NaCl/40% Sucrose	3.09	B	5.82	---	---
	15% NaCl/35% Sucrose	2.55	B	6.45	---	---

[1]Nine point Hedonic Scale (9=like extremely; 1=dislike extremely)
[2]Significance: within a test, samples having a different letter are different at a 1% level of significance
[3]Two step: 1 h in 25% NaCl, rinsed, 3 h in 60% sucrose, rinsed

It is seen that high organoleptic scores were attained with the pure sucrose and mixed carbohydrate solute systems. The salt-based systems were unacceptable even when the initial salt osmosis was followed by a sucrose osmosis step. The scores from the first test indicate that the pure sucrose systems were rated somewhat higher than either mixed system. This may reflect a desirable taste effect associated with the sweetness of sucrose. Tests on the effect of total solids content for the mixed carbohydrate solutions indicate that over the range of concentrations considered, the product was uniformly highly acceptable. Texture for all samples was well rated.

CONCLUSION

Osmotic dehydration is a successful method of preconcentrating fruit pieces prior to freeze-drying. All osmosis solutions at 60% solids are effective, so that the final choice of the solute system can be made on the basis of organoleptic and economic factors.

Lactose is suitable for use as a partial substitute for sucrose in both

dry and aqueous media, although, with its low solubility limit (about 25%), lactose cannot be used alone. In dry systems, a caked layer of lactose forms a barrier around the fruit piece, preventing further transport of water from the sample. Mixing lactose and sucrose alleviates this problem. Organoleptic evaluations have shown high acceptability for apples treated in sucrose-lactose systems. Maltodextrin can also be used as a partial substitute for sucrose. Both maltodextrin and lactose have low levels of sweetness, making them desirable as osmotic agents for food materials requiring less sweetening.

Based on osmosis kinetics, 25% sodium chloride is by far the best osmotic agent. This is undoubtedly due to its higher molar concentration for a 25% weight concentration and its ionization in solution. Even when combined with sucrose, high osmosis rates were observed, but the ability to use sodium chloride is limited in processing fruits due to its saltiness. Organoleptic evaluations of apples processed with sucrose-salt mixtures show them to be acceptable, but salt may be suitable for dehydration of other food materials.

Moderate agitation of the osmosis solution and the apples is desirable, especially at higher solids concentrations. The use of vacuum contacting of solution with fruit pieces gives higher increases of solids content due primarily to uptake of osmosis solute. This occurs since air removed from apple tissue by the evacuation leaves voids that are filled by the solution. It has been observed that the apple sugar content is so elevated that during freeze-drying, there is some collapse of the sample. The dried product is not as crisp (more chewy), as those produced by the agitation system. This may be acceptable to certain food items though apples are preferred when crisp.

ACKNOWLEDGEMENT

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COMPRESSION OF FOOD DURING FREEZE-DRYING[1]

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ABSTRACT

Compressed freeze-dried foods offer significant advantages in reduced volume to be transported, stored, and reduced packaging material requirements when compared to the noncompressed products. To prevent fragmentation during compression, it is necessary that the product have sufficient plasticity. This is currently accomplished by remoistening the freeze-dried food prior to compression. The results reported here demonstrate that sufficient plasticity exists at the ice interface, so that foods can be compressed during freeze-drying. The degree of compression is directly related to compression pressure. Compression during freeze-drying does not influence product moisture content and gives a more rapidly rehydrating product. The extent of compression has little effect on the extent of rehydration.

INTRODUCTION

Freeze-drying has long been recognized as the method of dehydration producing products having the best quality and retained nutritional value and is now beginning to emerge as an important process for

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dehydration of foods. When foods undergo freeze-drying, very little change in the physical shape of the material occurs. While the retention of physical shape has long been viewed as desirable since it indicates no structural change in the product, it does result in an open and porous structure which gives a very light and fragile product of low packaging density. The disadvantages related to low packaging density can be summarized as:

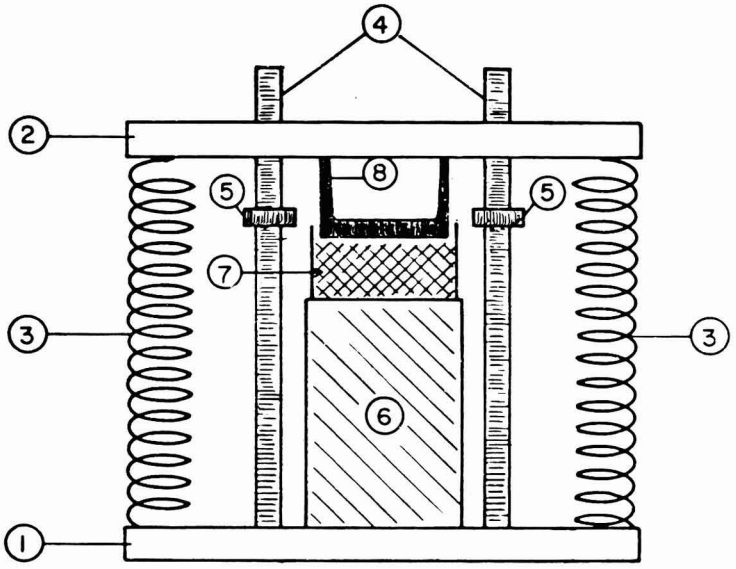
(1) The open structure is very susceptible to oxidation and moisture pickup, and thus special packaging is often required.
(2) Large package volumes are required to enclose small product weights.
(3) Readily friable products produce fines during handling and transportation which is essentially wastage of the products.
(4) Large storage space is required.

A more efficient food system will result by eliminating or minimizing the above mentioned effects. This can be accomplished by compressing the food to remove the void volumes which have been produced by sublimation of ice crystals during the drying process. To accomplish this compression of the product, it is necessary to have the food solids in a plastic state so that when high compression pressures are applied, fragmentation is avoided. This plastic state can be achieved by bringing the food to a relatively uniform moisture content in the range of 5—20% depending on the food product (Hamdy 1960). For some products of high sugar content (such as fruits), the plastic state can be achieved by manipulation of temperature alone (Rahman *et al.* 1970). The humidified or heated foods are placed in a cell and subjected to high pressures by means of a hydraulic press and then redried or cooled, yielding the compressed product. For the case of low sugar products, this process involves a number of additional processing steps requiring handling and time.

Studies on determination of moisture profiles during freeze-drying have shown that the ice-dry layer interface (or the diffusion zone) may be as much as a few millimeters (mm) thick and has an average moisture content of 10—20%, not considering the ice at the interface (Bralsford 1967; Gentzler and Schmidt 1973; Aquilera and Flink 1974).

This study investigated whether the moisture gradients which appear to be present at the interface during freeze-drying will give sufficient plasticity to allow compression of the product during freeze-drying. Success of this approach could eliminate the two current approaches: overdrying and subsequent rehumidification of the food to the plastic range, or long drying times and controlled freeze-drying aimed at giving samples a uniform final moisture content of about 10—20%.

METHODS



1 Base Plate
2 Top Plate
3 Matched Set of Springs (Extension Type)
4 Threaded Guide Rods
5 Nuts for Raising Top Plate
6 Sample Cell Stand
7 Sample Holder
8 Compression Plate

FIG. 1. SCHEMATIC OF COMPRESSION CELL

Figure 1 shows a schematic diagram of the device which was used to compress foods during freeze-drying. The force which results from extension of springs (3) is used to attain compressive pressures high enough to compress foods.

During the compression process, the compression force will decrease, since the elongation of the springs will be reduced. This change in force can be minimized by choosing springs which (1) have a large initial tension (the force required to initiate extension); (2) are long, so that the change in length (ΔL) compared to the rest length (L) is small for a given sample thickness; and (3) have a reasonable relationship of modulus

($\Delta F/\Delta L$) to initial tension, so that a change in force (ΔF) because of a change in length (ΔL) is small compared to initial tension. In practice the compression cell (Fig. 1) is assembled without the sample holder (7). The nuts (5) are used to raise the top plate (2) and maintain the springs (3) under tension. The frozen material in the sample holder (7) is placed on the sample cell stand (6). The compression plate (8) is inserted, and the nuts (5) are lowered allowing the top plate (2) to contact the compression plate (8). The nuts are lowered further, so that the top plate (2) can move down the guide rods (4) without hitting the nuts before the end of the compression and freeze-drying process. The sample holder (7) is constructed of aluminum and stainless steel mesh to allow application of the compressive force without significantly impeding the flow of vapor from the drying surface.

The overall experimental procedure is shown in Fig. 2. The sample is prepacked in the sample holder and frozen at −20 °C. Before loading the sample holder into the compression cell and applying the compressive force, the sample is further cooled by a stream of liquid nitrogen to ensure sufficient cooling during the cell assembly and transfer steps.

The spring extension is measured prior to insertion in the freeze-drier and following removal from the freeze-drier. From the force-extension calibrations made for each set of springs, these measured lengths are used to calculate the initial and final forces exerted on the product by the springs.

Identical samples were placed in a glass beaker and freeze-dried without compression at the same time. Freeze-drying was generally conducted at pressures below 0.15 torr and with heat input governed by the ambient conditions. Following freeze-drying, samples were evaluated for compression ratio (initial volume divided by final volume) and rehydration behavior.

Samples were rehydrated at different water temperatures — namely, cold (∼40 °F), warm (∼90—100 °F), and hot ∼130—160 °F). Individual pieces were rehydrated, and their weights measured at intervals over a 30-min period after the initial immersion. In a few cases with beef cubes, rehydration was continued for up to one hour. During the rehydration period, the samples were immersed under water and held submerged with a screen except for the time when weights were recorded. Prior to weighing, the samples were allowed to drain for ten seconds, and the surfaces lightly blotted. In an attempt to improve the extent of rehydration, vacuum treatments prior to rehydration were conducted by two methods. In one method the samples were evacuated by means of an aspirator for a few minutes; the vacuum was released with air, and the samples were then rehydrated. In the second method the vacuum was released with water instead of air.

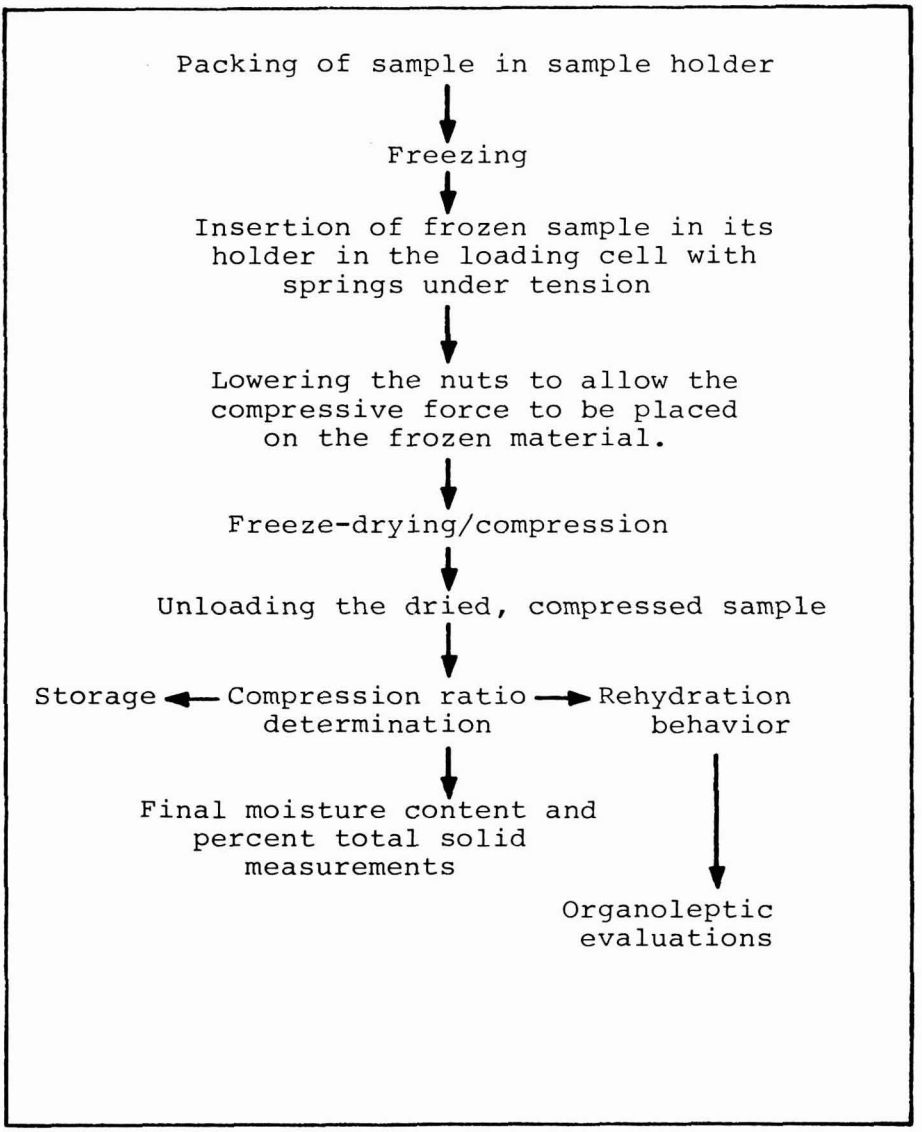


FIG. 2. FLOWSHEET OF EXPERIMENTAL PROCEDURES

Moisture content was determined on 1—2 g of freeze-dried sample by drying with an IR lamp to a constant weight and reporting as percent M.C. = grams water × 100/one g dry sample.

RESULTS AND DISCUSSION

A variety of fruit, vegetable, and meat products were tested with this compression system. Results for cut green beans, carrot cubes, beef cubes (1.5 × 1.5 × 1.5 cm), and shrimp (pieces) are discussed below while Table 1 gives results for some of these products at selected comparative compression pressures.

The range of compressive pressures used were between 42 and 185 psi. These values are significantly lower than the pressures (100—5,000 psi) used with the currently available compression method, especially when compared at corresponding degrees of compression. Besides differences in plasticity, this may be because of the differing thicknesses of the samples to be compressed. In general, with techniques involving rehumidification of an already dried sample, bed heights of several centimeters are compressed in one step, whereas the plastic zone at the ice-dry layer interface is only a few millimeters thick at the most; and it can be expected that much lower pressures are required to compress this narrow zone.

For a given thickness of each sample, the compression ratio was linearly related to compressive pressure over the range of pressures tested. However, the actual compression value at a given force was related to the particular food item. Figure 3 illustrates the relationship of compression ratio and compressive pressure for green beans. The term compression ratio is defined as the ratio of the initial volume of the sample to its final volume following compression and drying. The numerical value of the compression ratio will vary depending on the method of measuring or defining volume. For example, the "displacement" compression ratio is determined in a manner which is similar to the technique for measuring the volume of bread using rape seeds. The volume reduction of the individual piece is measured and thus no void volume for the bulk sample is included.

In measuring "packed" compression ratio, initial and final volume of the packed sample as occupied in the sample holder is determined. Since the sample in the holder is compressed only in one direction (i.e. length and width do not change), the ratio of the initial height to the final height is the measure of this compression ratio and is thus also labeled as compression ratio by height.

For determination of the random compression ratio, the initial volume is defined as the volume which is occupied by the given sample when randomly placed in its normal container or package. This volume is much larger than when the sample is orderly packed in the sample holder. Values given in the literature are usually based on random compression ratio.

Table 1. Properties of freeze-dried food products compressed at selected pressures

Product	Average Compression Pressure (psi)	Compression Ratio		Final Moisture (% wt)		Extent of Rehydration After 30 Min[1] (% of Fresh)	
		Height	Random	Compressed	Noncompressed	Compressed	Noncompressed
Green Beans	42	3.3	5.6	2.6	2.5	87	63
	61	3.6	6.1	4.4	4.3	64	50
	98	5.5	9.2	3.6	2.6	91	59
Carrots	64	1.5	2.7	3.4	2.2	77	80
	97	3.0	5.3	2.8	1.8	85	66
	126	4.2	7.5	3.8	5.4	72	78
Beef Cubes	63	1.4		2.6	2.4	82	90
	125	2.0		1.1	1.1	84	94
	185	2.7		1.1	1.0	68	92

[1]Rehydrated in cold water without vacuum treatment

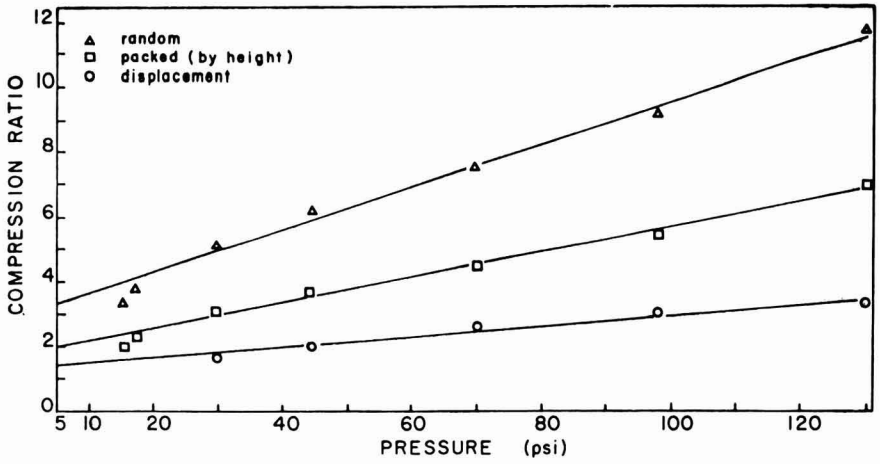


FIG. 3. EFFECT OF COMPRESSIVE PRESSURE ON COMPRESSION RATIO FOR GREEN BEANS

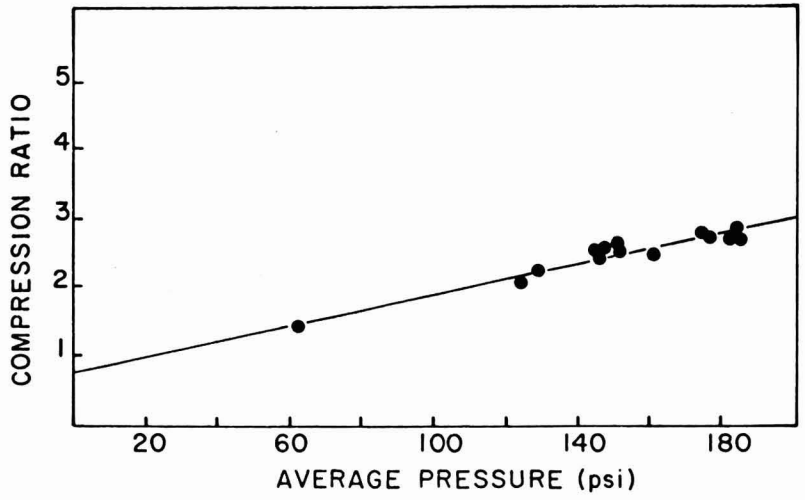


FIG. 4. EFFECT OF COMPRESSIVE PRESSURE ON COMPRESSION RATIO (BY HEIGHT) FOR BEEF CUBES

Theoretical consideration would indicate that compression ratio will reach a maximum value at some compressive pressure so that no increase in compression ratio occurs with further increase in compressive pressure. It is thought that this maximum compression ratio is directly related to the initial water content of the food. Besides elimination of the void volumes between the individual pieces, compression can also result

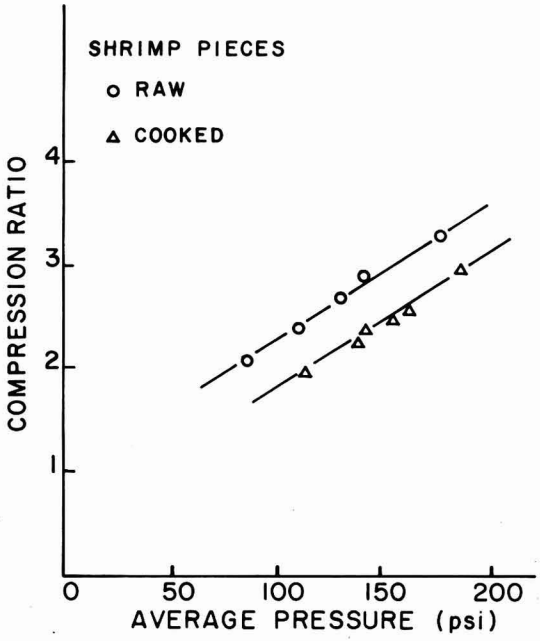


FIG. 5. EFFECT OF COMPRESSIVE PRESSURE ON COMPRESSION RATIO (BY HEIGHT) FOR RAW OR COOKED SHRIMP PIECES

in elimination of the voids that are produced by sublimation of ice crystals. The loss of void volume associated with sublimation of ice reflects the true change in the sample volume and will determine the maximum displacement compression ratios attainable for a reversible compression. For example, lean beef has approximately 65—70% water. Because of the close packed nature of the beef cubes, the packed and displacement compression ratio values are essentially identical. The maximum compression ratios for beef obtained in experiments reported here (Fig. 4) or in the literature (Brockmann 1965) for ground beef products are approximately 3:1, which corresponds to about 67% reduction in volume. Random compression ratios have not been calculated for beef cubes since this compression ratio is directly dependent on the piece size; however, for the size of the beef cubes used in this study, a factor of ~1.5 may be used to convert compression ratios by height to random compression ratios.

While the food can be visualized as a plastic material with a constant

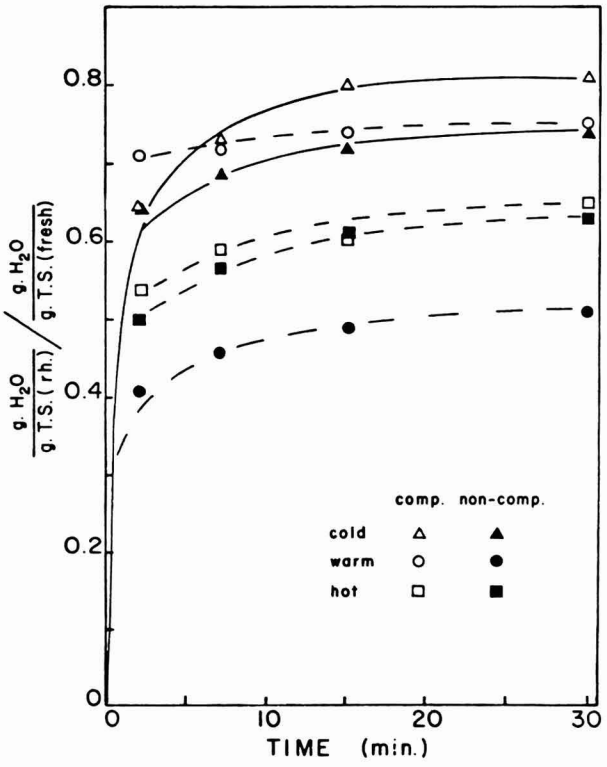


FIG. 6. EFFECT OF TIME AND WATER TEMPERATURE ON THE REHYDRATION BEHAVIOR OF COMPRESSED AND NONCOMPRESSED GREEN BEANS

deformation modulus, since when stress is applied the extent of compression is linear with applied force, there is a minimal resistive force that has to be overcome before compression initiates. This stress which is relatively high with beef cubes, shrimps, and carrots (>50 psi) and low with green beans (<10 psi) apparently is dependent on the physical characteristics of the particular food.

In experiments with shrimp the response of the compression ratio to the change in compressive pressure was similar for both raw and cooked samples (i.e. similar slopes of compression ratio-compressive pressure lines). Figure 5 shows that the only difference seems to arise from a higher yield stress which must be applied to cooked shrimp to initiate compression. It should be noted that the cooking process results in a loss

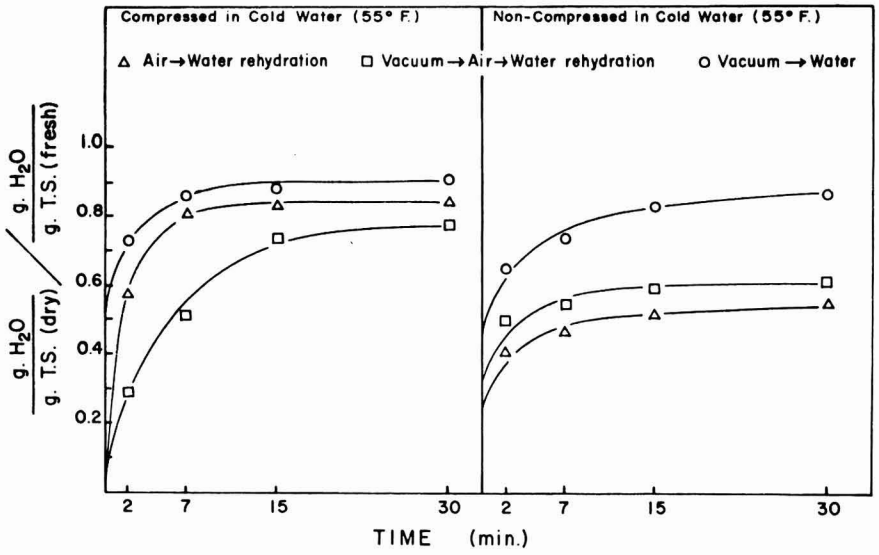


FIG. 7. EFFECT OF AN EVACUATION TREATMENT ON SUBSEQUENT REHYDRATION BEHAVIOR OF GREEN BEANS

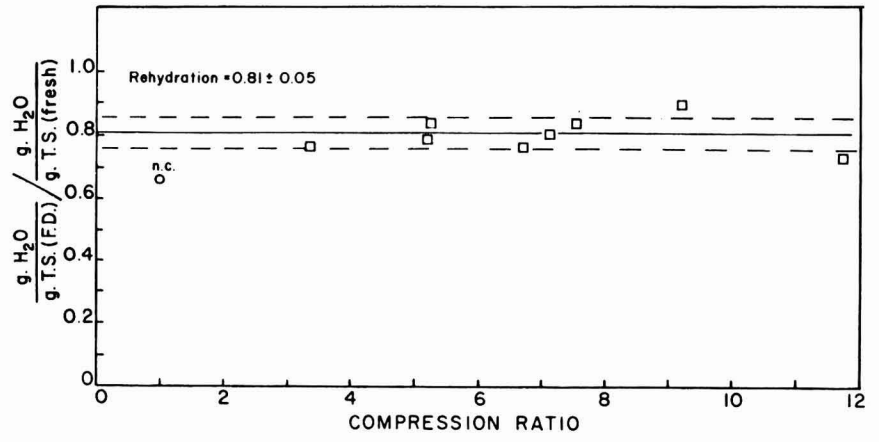


FIG. 8. EFFECT OF COMPRESSION RATIO ON EXTENT OF REHYDRATION FOR GREEN BEANS

of some shrimp fluid accompanied by a 10—20% reduction in volume of the pieces. It was noted that the final volumes of the compressed cooked

and raw samples were the same at a given applied pressure. This would indicate that compression of the shrimp pieces at any compressive pressure had continued until a force equilibrium existed between the applied force from the springs and the resistive force of the food. It appears that the sole effect of cooking was to eliminate some fluid from the shrimp pieces with a reduction in volume without changing the compressive rheological properties of the sample. It is well known that cooking changes the textural properties of foods, and thus further studies are needed to confirm the above hypothesis of unchanged rheological behavior.

Final moisture contents of compressed samples were always similar to those of noncompressed controls freeze-dried at the same time. In most cases, the moisture contents were between 1.5—5% (Table 1), which shows that compression during freeze-drying does not prevent dehydration to low moisture contents. Final moisture content did not depend on the compression ratio.

Rehydration of the compressed samples was quick and in most cases went to equilibrium within 15 min (Table 1). Samples generally reattained their precompression volume after rehydration. Figure 6 shows the typical behavior for rehydration of green beans at different temperatures. Vegetables seemed to rehydrate best in cold water, whereas beef cubes rehydrated faster in warm and hot water. However, at the higher temperature rehydration conditions, the color of the meat became dark and had a cooked appearance. Cold water rehydration, though slower, gave a better quality meat product. Freeze-dried noncompressed vegetables were observed to generally rehydrate to a lesser extent than compressed samples when just immersed in water (Fig. 7). Rehydration was then conducted by evacuation of the freeze-dried samples prior to the addition of water. The vacuum was broken with air or the rehydration fluid. The effect is noted for compressed samples, whereas the noncompressed samples showed a marked increase in rehydration when a vacuum is used. Vacuum rehydration increased the extent of rehydration of noncompressed vegetables to that of the compressed samples. These results indicate that the cause for lower uptake of water by noncompressed vegetables was because of air entrapped in the cells and that some structural changes which are caused by compression affect either removal of entrapped air in the cells or water uptake by the cells resulting in improved rehydration behavior.

The extent of rehydration of vegetables did not seem to be affected by the degree of compression. This is shown for green beans in Fig. 8. However, beef cubes showed a slight trend toward reduced rehydration with increasing compression ratio (Fig. 9). The narrow range of compres-

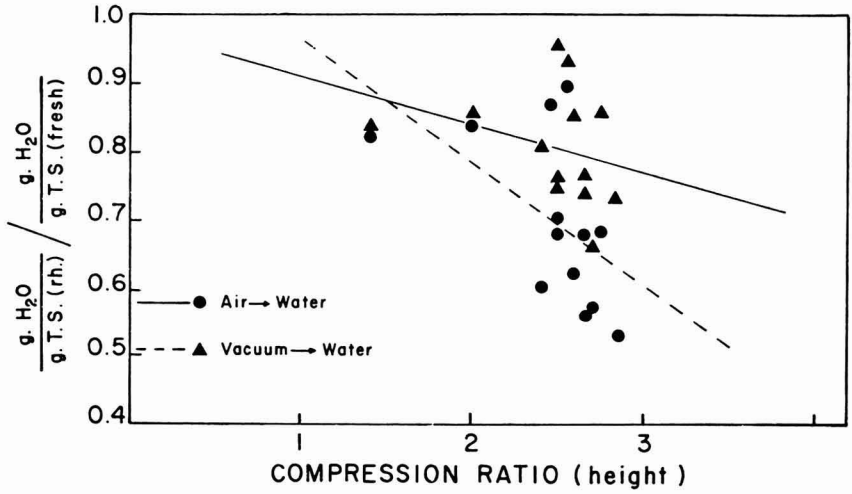


FIG. 9. EFFECT OF COMPRESSION RATIO ON EXTENT OF REHYDRATION FOR BEEF CUBES

sion ratios for beef cubes makes it difficult to develop a definite relationship for the influence of degree of compression on rehydration for this product. It was also noted for compressed beef that vacuum rehydration generally gave improved rehydration.

The results indicate that compression of foods during freeze-drying is feasible and can be considered as an alternative method for preparation of compressed freeze-dried foodstuffs.

ACKNOWLEDGEMENT

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NUTRITIONAL ASPECTS OF FOOD IRRADIATION: AN OVERVIEW

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ABSTRACT

When foods are exposed to ionizing radiation under conditions envisioned for commercial application, no significant impairment in the nutritional quality of protein, lipid and carbohydrate constituents was observed. Irradiation was no more destructive to vitamins than other food preservation methods. Protection of nutrients is improved by holding the food at low temperature during irradiation and by reducing or excluding free oxygen from the radiation milieu.

INTRODUCTION

When foods are exposed to ionizing radiation under conditions envisioned for commercial application, the effects upon nutrients are not markedly different in degree from those observed with other food preservation methods (Josephson *et al.* 1975; Josephson *et al.* 1974). Because of the protective qualities inherent in foods, the sensitivity of nutritional components is less than that of the same nutrients irradiated in pure form or in artificial solutions and mixtures (Bregvadze and Bokeriya 1971; Metlitskii *et al.* 1968). Protection of nutrients is improved if the food is held at low temperatures in an oxygen-free medium during the irradiation process (Thomas and Josephson 1970; Metlitskii *et al.* 1968; Kharlamov and Shubnyakova 1964; Southern and Rhodes 1967).

This report concerns itself with the nutritional aspects of foods exposed to ionizing radiation under conditions being considered for application in commercial operations.

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[1]Deceased.

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Kennedy (1965) observed little change in nutritive value of animal feeds (protein concentrates) when 0.5 and 1.0 Mrad doses were applied — and no nutritional changes with frozen eggs irradiated at 0.5 and 5.0 Mrad. Ley (1972, 1975) noted excellent results with radappertized feed for germ-free rat and mouse colonies which were maintained for 5 years. Radappertization and radicidation have been used in preference to thermal sterilization to sustain germ-free and specific-pathogen-free rats, mice, pigs and chickens (Sato 1970; Schoen and Hiller 1971; Udes *et al.* 1971; Ley *et al.* 1969; Coates *et al.* 1963). Others have reported that both the biological value of proteins, and the metabolizable energy value, of composite rodent diets are unaltered by radappertization at 5.6 Mrad (Raica and Howie 1966; Read *et al.* 1961; Kraybill 1960).

CARBOHYDRATES

Radiation's main effects upon carbohydrates are those of hydrolysis and oxidative degradation. Polysaccharides are depolymerized, and cellulose is made more susceptible to enzymatic hydrolysis. In short, radiation energy converts complex carbohydrates into simpler compounds. Although irradiation may cause changes in the physical and chemical properties of high-carbohydrate foods such as grains and vegetables, these changes have not been shown to have any nutritional significance.

A sterilization dose of 5.58 Mrad was shown not to affect the availability of the carbohydrates of 8 vegetables, fruits, and cereal products (Read *et al.* 1961). Rat feeding tests carried out by Lang and Bässler (1966) showed no differences in the utilization of the starch of irradiated (10 Krad and 100 Krad) and nonirradiated potatoes. SaintLèbe *et al.* (1973) obtained similar results with dry maize starch exposed to a radiation dose of 600 Krad.

LIPIDS

Ionizing radiation's effects on lipids are not unlike those wrought by oxidative and heat processes. Irradiation of lipids results in a large number of compounds, the exact number depending upon the fatty acid composition (Mitchell 1957; Partmann 1962; Chipault 1962; Merritt 1966; Nawar 1972). The main reactions involve oxidation, polymerization, decarboxylation, and dehydration. Unsaturated fatty acids are more readily oxidized than are the saturated acids. The chemical changes

are minimized by irradiating the products when they are frozen, and by packaging them so as to exclude light and oxygen (Gel'fand 1970).

When human subjects were fed pork irradiated to 2.8 Mrad and stored for 1 year at room temperature, identical apparent digestibility values were obtained for irradiated and nonirradiated fat (Plough *et al.* 1957). However, lard irradiated to 5.58 Mrad was absorbed more slowly than nonirradiated lard, due to delayed emptying of the stomach contents (Schreiber and Nasset 1959). Nevertheless, overall digestibility was unaffected, indicating that lipolysis and absorption of end products were not seriously distributed by feeding irradiated lard.

PROTEINS AND AMINO ACIDS

Although very high levels of irradiation have marked effects on proteins and amino acids, levels envisaged for use in food processing show only a minimum of deleterious effects. Ley *et al.* (1969) reported that radappertized protein supplied in a rat diet in the form of soya, meat-and-bone meal, and fishmeal showed that irradiation — even at a 7.0 Mrad dose — has no significant effect on digestibility, biological value, and net protein utilization (Table 1), or on amino acid composition (Table 2).

Table 1. Effect of irradiation on the protein quality of rat diet

Dose (Mrad)	True Digestibility	Biological Value	Net Protein Utilization
0	85.6	80.5	68.9
0.5	83.6	75.8	63.5
1.0	86.5	81.7	70.6
2.5	87.0	78.1	68.0
3.5	84.8	77.3	65.4
7.0	85.3	76.4	65.2

Source: Ley *et al.* (1969)

The United States Army Medical Department, and its contractor, Industrial Bio-Test, Inc., have been involved since 1971 in a very comprehensive study designed to assess the safety for consumption ("wholesomeness") of beef heated to inactive autolytic enzymes and radappertized by exposure to the gamma rays from ^{60}Co and electrons (10 MeV maximum energy), in a 4.7-7.1 Mrad dose range, at temperatures between −40 and −9 °C. Heat-sterilized beef and frozen beef (both nonirradiated, but enzyme-inactivated in the same fashion as the ^{60}Co-and electron-irradiated beef) served as the controls. As the data in Table 3 in-

Table 2. Effect of a radiation dose of 7.0 Mrad on the amino acid composition of the protein in rat diet

Amino Acid	Unirradiated Diet (g/16 g N)	Irradiated Diet (g/16 g N)
Asparagine	8.85	8.38
Threonine	3.80	3.73
Serine	4.17	4.16
Glutamic Acid	15.70	15.61
Glycine	5.82	5.79
Analine	5.61	5.54
Valine	4.78	4.68
Isoleucine	3.99	3.99
Leucine	7.44	7.47
Tyrosine	3.28	3.38
Phenylalanine	4.12	4.28
Lysine	5.72	5.82
Histidine	2.29	2.37
Arginine	6.04	6.05
Methionine	2.33	2.11
Cystine	1.34	1.44
Tryptophan	1.16	1.32

Source: Ley *et al.* (1969)

Table 3. Effects of different processing methods upon cystine, methionine, and tryptophan content of enzyme-inactivated beef (WT %)

		Treatment			
Amino Acid	Storage (Months)	Frozen Control	Heat Sterilized (F_0=5.8)	60_{Co} (4.7-7.1 Mrad)[1]	Electron (10 MeV, 4.7-7.1 Mrad)[1]
Cystine	0	0.28	0.29	0.26	0.28
	15	0.27	0.25	0.27	0.30
Methionine	0	0.53	0.58	0.57	0.59
	15	0.54	0.54	0.54	0.56
Tryptophan	0	0.25	0.26	0.25	0.26
	15	0.24	0.23	0.22	0.23

[1]Packaged beef air-evacuated to internal pressure (IP) of approximately 100 mm Hg. IP at start and after irradiation and thawing was approximately 250 mm and 350 mm Hg respectively. Temperature of product was −40 to −5 °C during irradiation.

Source: Office of the Surgeon General, Department of the Army, Contract No. DADA 17-71-C-1030, Industrial Bio-Test, Inc., Contractor.

dicate, there was no significant destruction of cystine, methionine, and tryptophan, the three amino acids considered most sensitive to ionizing radiation, immediately after radappertization, and after 15 months' storage of the radappertized beef at room temperature.

De Groot *et al.* (1972) studied the nutritive value of radiation-pasteurized chicken. Data excerpted from their report (Table 4) show that

Table 4. Amino acid analyses of irradiated and nonirradiated chicken, stored for 6 days at +5 °C and cooked

	Amino Acid Content (g/16g N)		
Amino Acid	0 Krad	300 Krad	600 Krad
Isoleucine	4.2	4.2	4.3
Leucine	6.7	6.7	6.8
Lysine	7.1	6.9	7.1
Methionine	2.3	2.3	2.35
Cystine	0.98	1.02	1.02
Phenylalanine	3.6	3.5	3.5
Tyrosine	2.9	2.8	3.0
Threonine	4.0	4.0	4.1
Tryptophan	0.98	0.93	0.96
Valine	4.8	4.8	4.9
Arginine	6.6	6.5	6.6
Histidine	3.4	3.3	3.3
Alanine	6.4	6.5	6.6
Aspartic Acid	8.4	8.2	8.4
Glutamic Acid	13.6	13.6	13.6
Glycine	8.5	8.8	9.0
Proline	5.5	5.6	5.7
Serine	4.1	4.1	4.2
Availability of Lysine	94%	95%	96%
$\frac{\text{Amino Acid N}}{\text{Kjeldahl N}} \times 100$	93	92	93

Source: de Groot *et al.* (1972)

Table 5. Nutritive value of protein in chicken stored at +5 °C for 4—7 days and cooked

Dose (Krad)	Protein Efficiency Ratio
0	2.18
300	2.34
600	2.21

Source: Derived from de Groot *et al.* (1972)

Table 6. Amino acid content of raw beef (g/100 g dry weight) exposed to accelerated electrons or gamma radiation (0.6 Mrad)

		Accelerated Electrons				$^{60}_{Co}$ Gamma Radiation
		2 MeV		4 MeV		1.17 MeV
Amino Acid	Control (Not Irradiated)	20 Krad/s	200 Krad/s	20 Krad/s	200 Krad/s	0.53 Krad/s
Cystine	0.72	0.71	0.87	0.65	0.62	0.86
Lysine and Histidine	15.42	13.46	15.07	14.29	13.79	14.95
Arginine	7.95	7.72	8.09	7.32	7.65	7.23
Aspartic Acid	7.04	6.85	6.65	6.41	6.78	7.15
Serine	2.82	2.97	2.60	3.04	2.96	2.79
Glycine	3.37	3.39	3.61	3.91	3.75	3.42
Glutamic Acid	11.82	11.75	11.11	12.04	11.72	11.50
Threonine	4.64	4.23	4.52	4.52	4.54	4.67
Alanine	4.64	5.10	4.95	5.12	5.19	4.82
Tyrosine	2.84	2.74	2.89	3.02	2.77	3.03
Methionine	2.48	2.38	2.46	1.91	2.30	2.52
Valine	5.35	5.21	5.08	5.71	5.63	5.15
Phenylalanine	4.10	4.57	4.90	4.69	4.96	4.15
Leucine and Isoleucine	9.19	10.04	9.74	9.96	9.93	9.32

Source: Derived from Frumkin *et al.* (1973)

a 600 Krad irradiation dose caused no significant changes in the amino acids in chicken stored at refrigeration temperature for 6 days and subsequently cooked and homogenized. Nor was the availability of lysine affected by the irradiation. Their protein efficiency ratio data on irradiated (600 Krad) and nonirradiated chicken stored at 5 °C for 4—7 days and then cooked (Table 5), as well as other data from their report, led them to conclude that "the nutritive value of the protein was not noticeably affected by the irradiation treatment of the chickens." Frumkin *et al.* (1973) applied radiation doses of 0.6 Mrad to raw beef (Table 6) and 0.8 Mrad to culinary meat (Table 7). They concluded that "the irradiation of raw and culinary treated meat to retard spoilage does not lead to a reduction in its protein nutritional value.

Table 7. Amino acid content[1] of stored culinary treated (ready-to-eat) beef preserved by exposure to ionizing radiation (0.8 Mrad)

			After 6 Months' Storage	
Amino Acid	Control (Deep Frozen)	After Irradiation	Control (Deep Frozen)	Irradiated
Lysine	8.98	8.95	8.12	8.81
Histidine	2.46	2.76	2.17	2.62
Arginine	5.41	5.20	4.97	5.36
Aspartic Acid	7.99	7.44	8.00	7.26
Threonine	3.70	3.82	4.38	3.60
Serine	3.19	3.34	3.67	3.10
Glutamic Acid	13.17	11.77	13.31	11.86
Proline	3.30	3.42	3.47	3.27
Glycine	3.57	3.94	3.65	3.72
Alanine	4.89	4.66	5.00	4.56
Valine	2.93	2.77	2.53	2.42
Methionine	2.10	1.88	3.01	1.30
Isoleucine	3.49	3.38	3.74	3.36
Leucine	6.30	6.12	6.87	5.33
Tyrosine	2.73	2.61	2.35	2.53
Phenylalanine	3.11	2.96	2.91	2.88
Tryptophan	1.37	1.47	1.32	1.35

[1]Expressed as % of protein.
Source: Derived from Frumkin *et al.* (1973).

Brooke *et al.* (1966) (Table 8) concluded that irradiation (250 Krad) of air-packed haddock fillets, followed by storage for 30 days in ice, and then steaming, had no deleterious effect upon the essential amino acids. As controls, Brooke and his co-workers used fresh raw nonirradiated haddockfillets. Similar results with claims irradiated at 450 Krad are also shown.

Table 8. Effect of irradiation on the essential amino acid content[1] of seafoods.

	Haddock Fillets		Clams	
Amino Acid	0 Krad[2]	250 Krad[3]	0 Krad[2]	450 Krad[4]
Isoleuine	4.64	4.76	3.75	4.00
Leucine	5.32	7.54	6.27	6.50
Lysine	10.78	9.40	6.89	6.69
Methionine	3.00	3.11	2.18	2.30
Phenylalanine	3.32	3.40	2.88	3.43
Threonine	4.50	3.72	3.49	4.05
Tryptophan	1.27	1.18	1.10	1.24
Valine	4.50	4.70	3.89	4.12

[1]Expressed as % of protein
[2]Fresh, raw
[3]Stored 30 days in ice after irradiation
[4]Air-packed and stored 30 days at 0°C after irradiation
Source: Derived from Brooke *et al.* (1964, 1966)

VITAMINS

Considerable reduction in sensitivity to irradiation can be obtained by keeping the product deep-frozen during irradiation. Thomas and Josephson (1970) compared the retention of thiamine, riboflavin, niacin, and pyridoxine in radappertized and thermally processed pork loin (Table 9). They found that irradiation at a low temperature was no more destructive to these vitamins than was heat processing. They obtained similar results with ham.

Table 10 summarizes results from the analysis of vitamins from three procurements of the previously mentioned enzyme-inactivated radappertized beef used in the U.S. Army's wholesomeness study. As in the pork study, irradiation was no more destructive to these vitamins than was heat sterilization.

De Groot *et al.* (1972) have studied the effects of irradiation on the vitamin content of cooked chicken (Table 11). They concluded that, with the possible exception of a slight decrease in vitamin E and thiamine contents at the 600 Krad level, there was no indication that the irradiation caused any vitamin destruction.

Dollar (1975) and Wenkham (1968) investigated the effects of irradiation levels of up to 250 Krad on the ascorbic acid content of papayas (Table 12). He stated that the most significant feature of his study was that ascorbic acid losses are delayed, and are not increased, by irradiation, and that reduced ascorbic acid was not oxidized by the radiation doses used in the study.

Table 9. Effect of different preservation methods on the vitamin content of shelf-stable canned pork loin.

Vitamin	Treatment	Mg/100G[1]	Retention (%)
Thiamin	Control	3.69 ± 0.22[2]	
	4.5 Mrad @ −80°C ± 5°	3.14 ± 0.25	85
	Thermally processed	0.76 ± 0.08	20
Riboflavin	Control	1.02 ± 0.28	
	4.5 Mrad @−80° ± 5°	0.79 ± 0.06	78
	Thermally processed	0.82 ± 0.02	81
Niacin	Control	20.3 ± 5.1	
	4.5 Mrad @ −80° ± 5°	15.9 ± 2.6	78
	Thermally processed	13.2 ± 1.8	65
Pyridoxine	Control	0.76 ± 0.05	
	4.5 Mrad @ −80°C ± 5°	0.75 ± 0.07	98
	Thermally processed	0.63 ± 0.07	84

Source: Thomas and Josephson (1970)

[1]Moisture, fat, salt-free basis

[2]Mean ± S.D.; three samples per treatment

Table 10. Effect of different processing methods upon thiamine, riboflavin, niacin and vitamin B_6 content of enzyme-inactivated beef (mg/kg)

		Treatment			
	Storage (Months)	Frozen	Heat Sterilized	^{60}Cobalt (4.7-7.1 Mrad)	Electron (4.7-7.1 Mrad)
Thiamin	0	0.97	0.63	0.83	0.77
	15	0.68	0.14	0.21	0.26
Riboflavin	0	2.80	2.63	2.83	2.60
	15	1.69	2.60	2.60	1.46
Niacin	0	48.6	48.1	48.8	46.8
	15	57.2	54.9	50.1	44.5
Vitamin B_6	0	2.50	2.13	3.93	5.20
	15	0.97	0.57	0.35	0.42

Source: Office of the Army Surgeon General (Industrial Bio-Test, Inc., Contractor)

The work of Dennison and Ahmed (1971—1972) and Wenkam and Moy (1968) has also shown irradiation of fruit to have minimal effect on ascorbic acid content (Table 13). Vitamin C retention in oranges, tangerines, tomatoes and papayas varied from 100 to 72% with irradiation doses of 40-300 Krad. Wenkam and Moy (1968) also reported that irradiation with 25 Krad had no significant effect on ascorbic acid or carotene content of mangoes.

Table 11. Effect of irradiation upon the vitamin content of irradiated cooked chicken

	Dose (Krad)	
	0	600
Vitamin A, IU/kg	2200	2450
Vitamin E, mg/kg	3.3	2.15
Thiamin, mg/kg	0.58	0.42
Riboflavin, mg/kg	2.10	2.25
Niacin, mg/kg	58.0	55.5
Vitamin B_6, mg/kg	1.22	1.35
Vitamin B_{12}, mmg/kg	21	28
Pantothenic acid, mg/kg	13	17
Folacin, mg/kg	0.23	0.18

Source: de Groot *et al.* (1972)

Table 12. Total ascorbic acid in papayas

Radiation Dose Krad	Days of Storage	
	0	7
	mg/100 g	
0	478	570
28	512	536
0	562	512
75	608	488
0	582	527
250	534	622

Source: Unpublished data (1975) Dr. Alexander M. Dollar, State of Hawaii Department of Agriculture, Honolulu

Wheat irradiated at either 20 or 200 Krad retained approximately 90% of its thiamine, riboflavin, and niacin (Vakil *et al.* 1973) (Table 14). Irradiation of bleached, enriched, hard-wheat flour in the range of 30—50 Krad had no detrimental effect on the thiamine, riboflavin, niacin, or pyroxidine content. Furthermore, the nutritive quality of bread made from this flour was unaffected (Heiligman *et al.* 1973).

Minimal losses of ascorbic acid result from irradiation doses of 5-15 Krad, which have been approved for commercial processing of white potatoes to prevent sprouting during storage. Doses as high as 12 Krad have little effect on the ascorbic acid content of onions (McKinney 1971).

Information is sparse on the effects of irradiation upon the so-called lesser-known vitamins. Vitamin B_{12}, para-amino-benzoic acid, pantho-

Table 13. Effect of radurization on ascorbic acid retention (%) in fruit

Product	Dose Krad	Retention Percent
Oranges, Temple	100	97
	200	72
Tangerines	40	104
	80	94
	160	94
Tomatoes	100	86
	200	86
	300	91
Papayas	125	110

Source: Calculated from Dennison and Ahmed)1971-1972) and Wenkam and Moy (1968)

Table 14. Effect of irradiation on vitamin retention (%) of wheat and wheat products

Krad	Thiamin	Riboflavin	Niacin	Pyridoxine
20[1]	88	91	88	———
200[1]	88	87	91	———
30-50[2]	100	100	89	100
30-50[3]	100	100	117	100

Source: Calculated from Vakil *et al.* (1973), and Heiligman *et al.* (1973).

[1]Wheat
[2]Flour
[3]Bread made from the irradiated flour

thenic acid, and folacin are all radiosensitive in aqueous solution. Such is not the case in food (Bregvadze and Bokeriya 1971; Metlitskii *et al.* 1968). Sheffner and Spector (1957) reported a considerable reduction in the radiosensitivity of vitamin B_{12} in raw whole milk. Irradiation of ground pork with doses of up to 5.58 Mrad resulted in less than 10% destruction of pantothenic acid and no destruction of folacin (Sheffner and Spector 1957). Richardson (1955) found no significant decrease in folacin activity in irradiated diets fed to chickens.

There is considerably less information about the effects of irradiation on fat-soluble vitamins than there is in regard to water-soluble vitamins. Goldblith and Proctor (1949) reported that carotene in solution was radio-

sensitive to cathode rays. Kung *et al.* (1953) showed that irradiation of whole milk with 400 Krad resulted in destruction of 40% of the carotenoids, 70% of the vitamin A, and 60% of the tocopherols. Further studies with dairy products indicate 31—68% losses of vitamin A. Carotene destruction during radiation treatment can, however, be minimized by the addition of ascorbic acid. Information is sparse because dairy products do not respond well organoleptically to irradiation, so no one is working in this area.

Vitamin D activity in chicks is decreased by gamma irradiation of the total diet with 2.79 Mrad at ambient temperature (Sheffner and Spector 1957). Vitamin E is radiosensitive; its estimated radiation destruction in whole milk was found to be 61% at a dose of 400 Krad (Kung *et al.* 1953).

Investigations by Richardson *et al.* (1956) showed no loss of vitamin K content in alfalfa leaf meal and fresh spinach irradiated to a dose of 2.79 Mrad at ambient temperature. No destruction of K_1, K_3, or K_5 was apparent in irradiated semisynthetic diets when they were fed to chickens.

CONCLUSION

The data presented here have been selected from the vast available literature on the basis of relevance to the nutrition of consumers. For this reason, less attention was given to the effects upon nutrient constituents irradiated in pure form, or in artificial solutions and mixtures. Wherever possible, the data under discussion reflected known processing parameters — which are directly germane to future commercial-scale applications. When food is preserved by ionizing radiation under processing conditions proposed for commercial production, nutrient destruction is no greater than that which occurs when food is preserved by more conventional means. This conclusion is based on data derived from *in vivo* studies, and from physical and chemical measurements.

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BACTERIAL SPORES: BIOPHYSICAL ASPECTS OF RECOVERY FROM RADIATION INJURY

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ABSTRACT

Differences in radiation resistance of 14 *strains of* Clostridium botulinum *spores could be correlated by computer analysis with differences in the lag or shoulder portion (L) of their respective radiation survival curves. The exponential decline portions (E) of the survival curves were nearly identical for all* 14 *strains. Autoradiographic, and diphenylamine assays indicated that strains* 33*A*, 62*A and* 51*B contained* 1, 2 *and* 2 *genomes per spore, respectively. However, no relationship could be detected between number of genomes and radiation resistance of the spores. Alkaline sucrose gradient sedimentation of* ^{3}*H-DNA indicated that L was characterized by production of DNA single-strand breaks (SSB). Radiation resistant strain* 33*A rejoined* 50—90% *of the initial SSB during irradiation or shortly thereafter, i.e. while the spores were still in the cryptobiotic dormant state. Rejoining of SSB seems to be due to high DNA-ligase activity in strain* 33*A. On the other hand, the radiation sensitive strain* 51*B showed no shoulder (L) and very little or no SSB rejoining. The exponential decline portion (E) seems to be associated with those lesions which cannot be repaired during irradiation or germination. It is thought that repair of these lesions is attempted after germination and initiation of metabolism and may involve DNA excision-resynthesis, and recombination. These repair mechanisms are error-prone and thus frequently result in cell death characteristic of the E portion of the survival curve.*

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INTRODUCTION

Problem

Effective food irradiation engineering demands thorough understanding of basic molecular aspects of cell injury and cell recovery. The present paper is concerned with bacterial spores, many of which are highly radiation resistant and at the same time present a potential public health hazard. Radiation may be employed to eliminate spores from irradiated food items as well as from medical devices, surgical implants, surgical and hospital supplies, biologicals and drugs. This paper analyzes two aspects of bacterial spores which appear to be of specific importance to radiation sterilization:

(1) The differences in radiation resistance among different strains of spore formers

(2) The relation of radiation resistance to the ability of spores to repair initial radiation injury.

The primary aim of this paper in relation to engineering and food is to correlate the available pragmatic knowledge of radiation biology of spores with modern understanding of molecular aspects of DNA repair and the special conditions and changes with respect to spore dormancy, germination, and outgrowth.

Background

Much information on radiation resistance of *C. botulinum* spores comes from multiple tube probability data and the inoculated pack method, respectively. From the point of view of theoretical understanding of underlying processes, survival curves are perhaps more meaningfully related to radiation resistance in terms of subcellular and molecular events in radiation damage, repair and recovery of spores.

In the present study we aimed to investigate this problem emphasizing typical Type A and B strains used previously for inoculated food pack experiments at the U.S. Army Natick Laboratory, and comparing them with the more radiation sensitive type E strains. It was considered essential to compare survival curves obtained in suspending media and under temperature conditions offering a minimum of radiation protection, such as generally used in theoretical laboratory experiments versus media and temperatures offering high protection i.e. comparable with conditions encountered in current food irradiation practices.

The molecular mechanism of DNA repair as related to radiation resistance has been studied using the alkaline sucrose gradient sedimentation technique.

MATERIALS AND METHODS

Fourteen strains or variants of serological types A, B, and E of *C. botulinum* were used in the present study. Microbiological methods were essentially those previously described (Grecz *et al.* 1977). In brief, heat-shocked spores were suspended in 0.067 M phosphate buffer, pH 7 and Pork Pea Broth (PPB), pH 7, respectively. The irradiation was accomplished with cobalt-60 at 0 °C and −190 °C, respectively. Temperature was controlled during irradiation as previously described (Grecz *et al.* 1965). The number of spores remaining viable after irradiation was determined by colony counts in Wynne's agar at 30 °C (Wynne *et al.* 1955). A computer program in FORTRAN, developed by Tyler and Dipert (1962) at Argonne National Laboratory in Chicago, was applied for estimation of the constants of the survival curves (Fig. 1), namely, the D_{10}, the length of shoulder (L), and the extrapolation number (N).

In subsequent experiments spores were irradiated in a cesium-137 Gammator (Radiation Machinery Corporation, Parsippany, N.J.). Tritium labelling of spore DNA, production of spore protoplasts, alkaline sucrose gradient sedimentation studies, and analysis of sedimentation data were essentially as previously described (Durban *et al.* 1974a; Durban *et al.* 1974b). Autoradiography of spore genomes was carried out by the method of Dennis and Wake (1966) and Kang and Grecz (1975, 1977).

RESULTS

Radiation Survival Curves of Spores of *C. botulinum* Strains

As an example, the basic effect of four irradiation conditions such as encountered in rich organic food media and at low temperatures on radiation survival curves of *Clostridium botulinum* 33A, 62A and 51B spores is shown in Fig. 2. The basic constants from these survival curves are compared in Table 1. The dose modification factors (DMF) for strain 33A indicate that PPB constituents at 0 °C had only a slight protection versus PO_4 buffer at 0 °C. However, in combination PPB and −190 °C were highly protective. As will be shown later radiation protection by PPB and −190 °C was primarily in the shoulder (L) of the radiation survival curve. Since L=O for the sensitive strain 51B, the DMF due to PPB and −190 °C was insignificant (Table 1).

The highly radioresistant strain 33A showed an extremely high extrapolation or target number N=39 to 90 (Table 3). Since N was historically linked with the number of nuclei, it was of interest to find the number of genomes per spore in 33A. Heavily labelled with ^{3}H-thymidine,

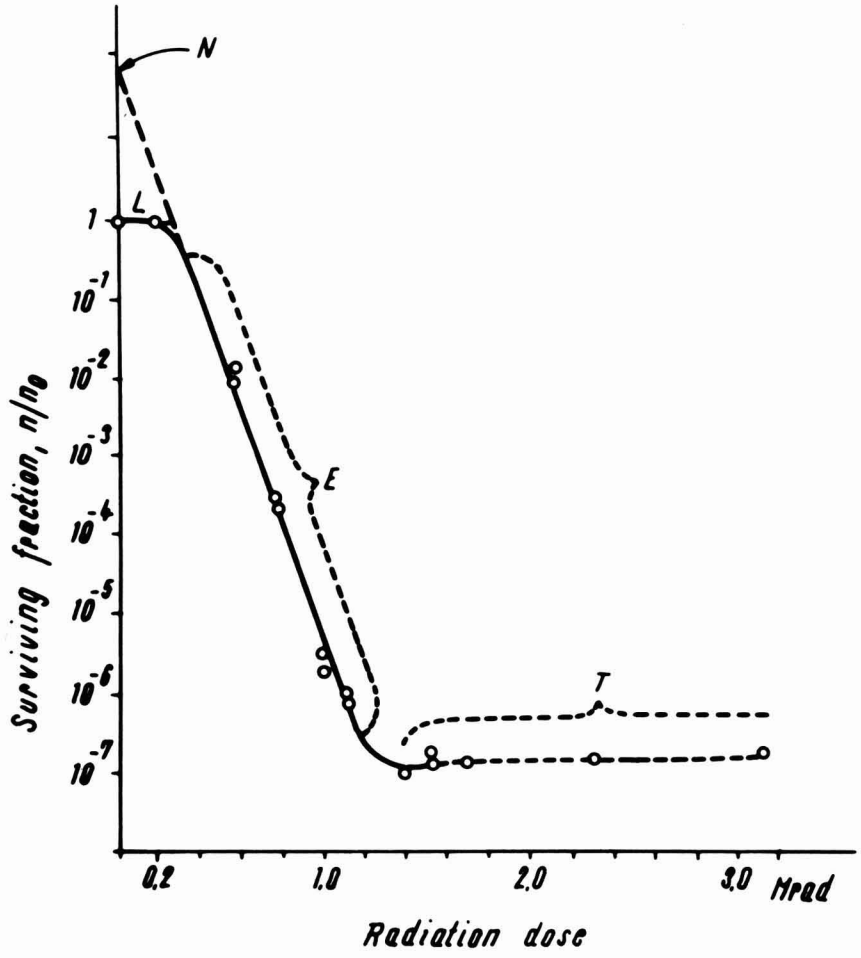

FIG. 1. ILLUSTRATION OF THE COMPONENT PARTS OF A SIGMOIDAL RADIATION SURVIVAL CURVE OF SPORES OF *CLOSTRIDIUM BOTULINUM* 33A (GRECZ AND TELATNYK UNPUBLISHED)

N = extrapolation or target number; L = lag or shoulder; E = exponential decline portion; t = tail portion. The exponential decline portion is characterized in this paper as D_{10} (exp), the 10% survival dose

spores were allowed to grow out in cold medium into cell chains and then subjected to autoradiography. Figure 3 shows in each cell chain only 2 grain spots, originating from the 2 DNA strands of the parent spore genome which segregates into daughter genomes. Assuming the semiconservative pattern of DNA replication during spore outgrowth,

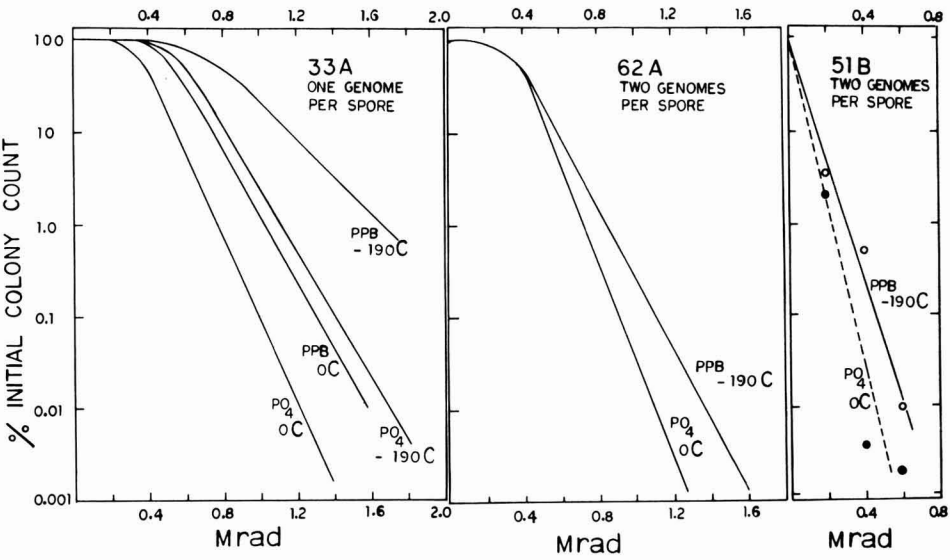

FIG. 2. ^{60}Co RADIATION SURVIVAL CURVES OF SPORES OF *CLOSTRIDIUM BOTULINUM* 33A, 62A AND 51B UNDER 4 DIFFERENT IRRADIATION CONDITIONS AS EVALUATED BY FORTRAN COMPUTER PROGRAM (TYLER AND DIPERT 1962)

PO_4 = 0.067 M phosphate buffer, pH 7. PPB = pork pea broth (Anderson 1951). The temperature during radiation was controlled either at 0°C or −190°C

Table 1. Basic constants for survival curves of *Clostridium botulinum* 33A and 51B under four irradiation conditions[a]

	N[c]	L[d] Mrad	D_{10}[e] (exp) Mrad	[b]DMF
C. botulinum 33A				
PO_4[b], 0°C	38.8	0.35	0.22	1.00
PO_4[b], −190°C	73.0	0.50	0.29	1.32
PPB, 0°C	76.2	0.50	0.26	1.18
PPB, −190°C	90.0	0.67	0.36	1.64
C. botulinum 51B				
PO_4[b], 0°C	0.9	0	0.12	1.00
PPB, −190°C	1.2	0.01	0.14	1.17

[a]This table gives computer processed data by the FORTRAN program of Tyler and Dipert (1962).

[b]PO_4 = Phosphate buffer, pH 7; PPB = pork pea broth (Anderson 1951). DMF was calculated as the ratio of a given D_{10} (exp) against that of spores irradiated in PPB, 0°C

[c]N = extrapolation of target number in the multitarget formula $n/n_0 = 1-(1-e^{-VD_1})^N$

[d]L = size of shoulder determined as illustrated in Fig. 1

[e]D_{10} (exp) = the 10% survival dose determined as illustrated in Fig 1.

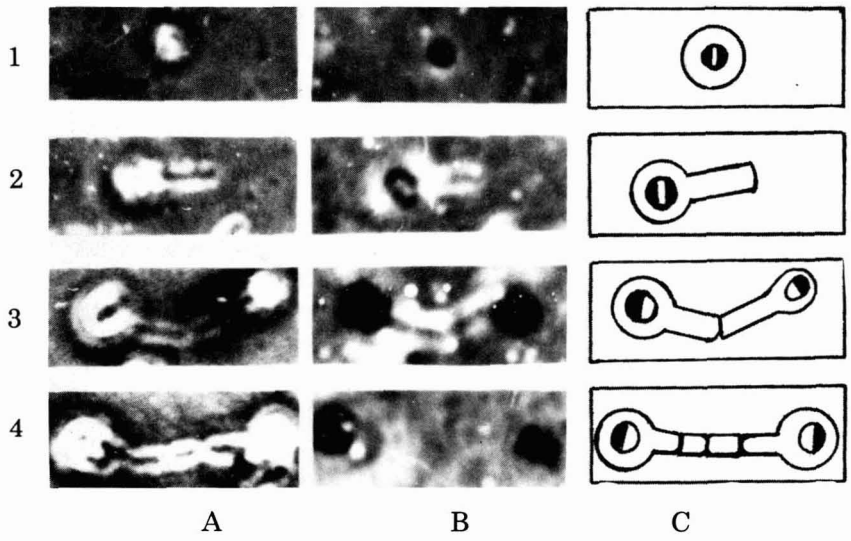

FIG. 3. AUTORADIOGRAPH OF *CLOSTRIDIUM BOTULINUM* 33A

The microscope was focussed on either the cells (A) or the film (B). Interpretation drawings (C): the initial chromosome (1) which is present in the spore remains in place while the spore germinates and grows out into a vegetative cell (2); after the first cell division, chromosome segregation occurs and the 2 original DNA strands (marked in black semi-circles) are distributed into the 2 daughter cells (3), and subsequently into the four-cell chain (4).

the results clearly demonstrate that each spore of 33A contains only one single genome. Therefore, the high N value in this strain must be due to factors other than multiple genomes.

This conclusion is further strengthened by the summary of recent data in Table 2 which demonstrates that the "multitarget" inactivation curves and the basic radiation resistance of spores of *C. botulinum* strains 33A, 62A, and 51B showed no obvious relationship to the number of genomes per resting spore.

The characteristic values of the survival curves of 14 strains and variants are summarized in Table 3. All strains except the very sensitive 51B exhibited sigmoidal survival curves with a distinct shoulder. It is readily apparent that differences in shoulder length (ca 60%) between individual strains were considerably greater than differences in D_{10} (exp) values (around 25%).

The correlation between the logarithm of the extrapolation number and the D_{10} or the L value is shown in Fig. 4. As seen from this Figure, log N changes approximately linearly as a function of the L values. The

Table 2. Basic characteristics of spores of *Clostridium botulinum*

Strain #	Extrapolation Number (N)		Radiation Resistance	Number of Genomes
	PO_4 buffer 0 °C	PPB −190 °C	D_{10} (Mrad)	
33A	38.8	90.0	0.334	1[a,b]
62A	72.0	19.6	0.224	2[a,b]
51B	0.9	1.2	0.129	2[b]

[a,b]Number of genomes per spore determined by autoradiography of ^{3}H-labeled genomes (a), or by the diphenylamine method (b). Autoradiography was carried out as described by Dennis and Wake (1966) and Kang and Grecz (1975, 1977). The diphenylamine method was as described by Burton (1956).

former correlation is apparently equivalent in both media in which spores were irradiated.

The dose mofication factor due to protective action of pork pea broth (−190 °C) on shoulder and D_{10} (exp) are calculated in Table 4. Pork pea broth (−190 °C) caused on the average a doubling of the shoulder length (DMF = 2.2), while the D_{10} (exp) was increased only very slightly (DMF = 1.3).

From these comparisons it may be concluded that (1) the slopes of the E portion of the survival curves of all strains were nearly identical; (2) that therefore, the factors responsible for slope E are most probably similar in all strains, and (3) that overall differences in radiation resistance of strains of *C. botulinum* are primarily due to processes related to the shoulder (L).

DNA Repair in the Shoulder of the Radiation Survival Curve

To gain an appraisal of the processes responsible for the shoulder (L), and the possibility of DNA repair in the cryptobiotic spore state, 2 strains of the same species, *C. botulinum* were chosen for further study: the highly resistant strain 33A, and the highly sensitive 51B (Table 3).

An example of alkaline sucrose gradient sedimentation patterns of DNA from unirradiated and anoxically irradiated spores of *C. botulinum* 33A and 51B receiving 0.3 Mrad are illustrated in Fig. 5. DNA extracted from irradiated spores sedimented at a slower rate than DNA from unirradiated spores. This indicated production of DNA strand breaks by γ-radiation. Furthermore, a very specific effect was exerted on the DNA sedimentation pattern by 0.02M EDTA, an inhibitor of direct rejoining of DNA strand breaks. In the case of strain 33A, a larger shift to more slowly sedimenting DNA fragments was observed in the presence of EDTA than in the absence of EDTA. In case of spores of strain 51B, the

Table 3. Characteristics of radiation survival curves of spores of *Clostridium botulinum* strains irradiated in phsophate buffer and pork pea broth, respectively

	Irradiated in PO_4-buffer, 0 °C				Irradiated in PPB, −190 °C			
C. botulinum Strain	N	Log N	L (Mrad)	D_{10} (exp) (Mrad)	N	Log N	L (Mrad)	D_{10} (exp) (Mrad)
33A	38.8	1.589	0.35	0.22	90.0	1.954	0.67	0.36
36A	2.6	0.415	0.07	0.17	3.4	0.531	0.14	0.27
37A	2.6	0.415	0.07	0.18	13.6	1.134	0.26	0.23
62A	72.0	1.857	0.35	0.19	19.6	1.292	0.34	0.26
12885A	8.6	0.934	0.22	0.23	30.0	1.478	0.46	0.31
9B	13.3	1.124	0.18	0.16	95.9	1.982	0.42	0.21
40B	2.1	0.322	0.08	0.24	10.8	1.033	0.27	0.26
41B	5.5	0.740	0.16	0.21	64.0	1.806	0.45	0.25
51B	0.9	−0.046	0	0.12	1.2	0.079	0.01	0.14
53B (fluffy)[a]	2.8	0.447	0.06	0.14	2.9	0.462	0.10	0.22
53B (lobed)	3.7	0.568	0.16	0.29	21.7	1.336	0.44	0.33
Type E, VH	12.7	1.104	0.14	0.13	28.4	1.453	0.25	0.17
Type E, Beluga	6.3	0.799	0.15	0.19	137.3	2.138	0.38	0.18
Type E, Alaska	16.7	1.223	0.21	0.17	3.2	0.505	0.12	0.24
Average ± Standard Deviation		0.82± 0.53	0.16± 0.10	0.19± 0.05		1.23± 0.64	0.31± 0.18	0.25± 0.06
Variation Coefficient		64.6%	62.5%	26.3%		52.3%	58.0%	24.8%

[a]Two variants of strain 53B, one forms fluffy colonies and the other lobed colonies
Survival indices N, L and D_{10} (exp) are explained in Table 1

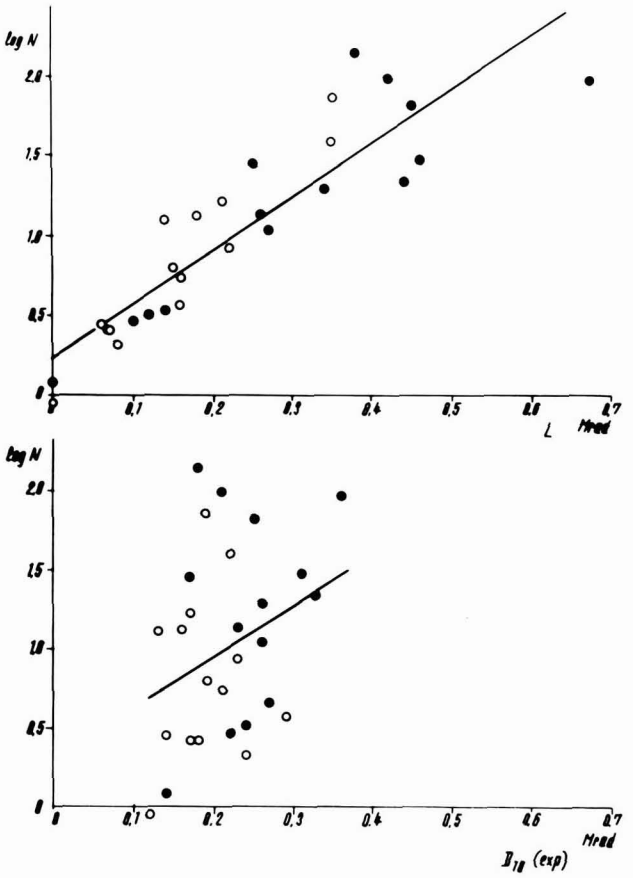

FIG. 4. THE CORRELATIONS BETWEEN THE LOG N AND D_{10} (EXP) VALUES AS WELL AS THE LOG N AND L VALUES, OF THE 14 *CLOSTRIDIUM BOTULINUM* STRAINS AND VARIANTS STUDIED

o = PO_4, O C; ● = PPB, = 190 C. Regression equations and determination coefficients: log N = 0.246 + 3.355 L; r^2 = 0.802; log N = 0.318 + 3.266 D_{10}; r^2 = 0.106.

presence of EDTA during irradiation had no effect on the sedimentation pattern, which seems to suggest absence of repair in this strain.

Table 5 summarizes the data and presents calculations from our alkaline sucrose gradient centrifugation studies. Of spores irradiated under anoxic conditions, the radiation resistant strain 33A sustained at least 6 DNA single-strand breaks (SSB) per chromosome when exposed to 0.3 Mrad γ-radiation in presence of the repair inhibitor, EDTA. In

Table 4. Dose modification factor (DMF) for shoulder (L) and exponential portion (E) for spores of *Clostridium botulinum* strains

Strain	DMF for L_a	DMF for D_{10} (exp)[a]
33A	1.9	1.6
36A	2.0	1.6
37A	3.7	1.3
62A	1.0	1.4
12885A	2.1	1.3
9B	2.3	1.3
40B	3.4	1.1
41B	2.8	1.2
51B	— —	1.2
53B (F)[b]	1.7	1.6
53B (L)[b]	2.8	1.1
VH-E	1.8	1.2
Beluga-E	2.5	1.0
Alaska-E	0.6	1.4
Average ± Standard Deviation	2.2 ± 0.9	1.3 ± 0.2

[a]Dose modification factor, DMF was calculated as the ratio of L or D_{10} (exp) under protective conditions in PPB (−190 C) versus L or D_{10} (exp) in phosphate buffer, 0 °C. (DMF = 1, means no difference between the two conditions)

[b]F and L for the variants of strain 53B indicate F = fluffy colony, L = lobed colony

Table 5. Induction of DNA SSB by γ radiation and their direct rejoining in spores of *C. botulinum* as determined by alkaline sucrose gradient centrigutation (McGrath and Williams 1966)

C. botulinum Strain	Treatment	#SSB[b] Per Chromosome
33A (radiation resistant)	Control	0
	0.3 Mrad	3
	0.3 Mrad + 0.2 M EDTA[a]	6
51B (radiation sensitive)	Control	0
	0.3 Mrad	28
	0.3 Mrad + 0.2 M EDTA[a]	27

[a]EDTA prevents rejoining of DNA SSB by withdrawing Mg^{2+} needed by DNA-ligase

[b]Number of DNA single-strand breaks (SSB) calculated from the formula $SSB = (D_1/D_2)^{2.63}-1$, where D_1 and D_2 are the average sedimentation distances of nonirradiated and irradiated spores, respectively (Town *et al.* 1971).

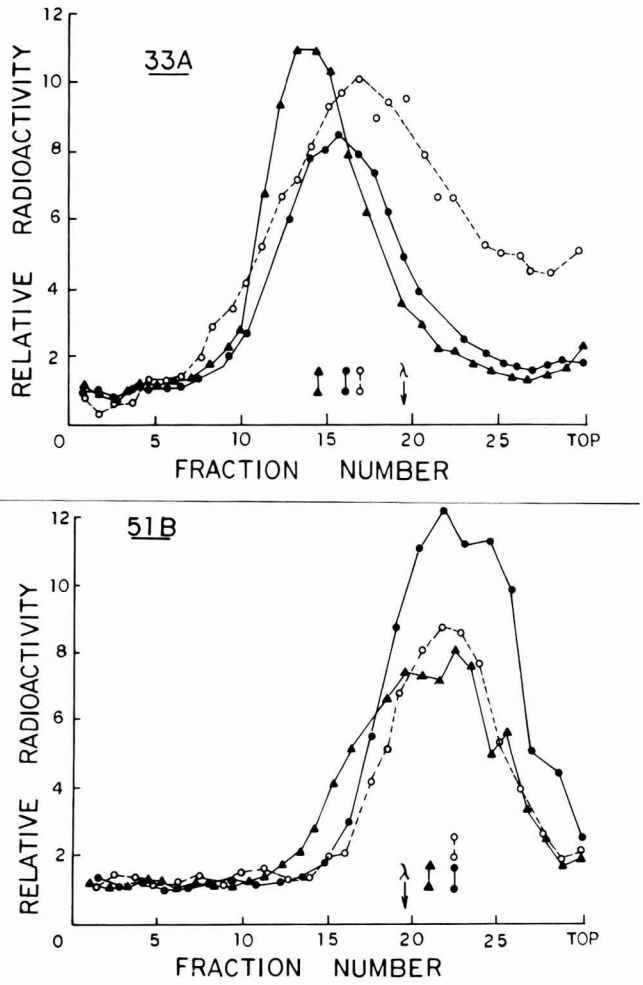

FIG. 5. SEDIMENTATION PATTERNS OF ^{3}H-DNA FROM DORMANT SPORES OF *CLOSTRIDIUM BOTULINUM* STRAINS 33A (TOP) AND 51B (BOTTOM)

▲ Non-irradiated controls with and without EDTA; ○ Irradiated with 0.3 Mrad, no EDTA; ● Irradiated with 0.3 Mrad with the spores suspended in distilled water containing 0.02 M EDTA (an inhibitor of DNA SSB rejoining). Vertical bars show position of sedimentation distance (D) of each sedimentation pattern. D was calculated from data using the formula of Kaplan (1966): $D = \Sigma X_i Y_i / \Sigma Y_i$ where Y_i is the fraction of radioactivity in the i-th fraction at distance X_i from the menicus. Since fractions were collected from the bottom and plotted from left to right, $X_i = 30$ minus fraction number i. Symbol λ shows the sedimentation distance (D) for lambda phage DNA (MW 3.1×10^7 for double stranded; MW $= 1.5 \times 10^7$ for single stranded DNA in the alkaline gradient).

absence of EDTA only 3 SSB were found indicating that 50% of the initial SSB were repaired. Radiation sensitive strain 51B sustained ca 28 SSB per chromosome when exposed to 0.3 Mrad, and no repair of SSB could be detected during radiation or immediately prior to germination. Presence of 0.0 2—0.03 M EDTA, prevented direct repair of DNA SSB in the radiation resistant strain 33A, but not in the irradiation sensitive strain 51B. A similar effect was obtained *in vivo* where EDTA present in the spore suspension during irradiation decreased survival of 33A by ca 30% in the shoulder portion of survival curve (See 0.2 Mrad in Fig. 6) but had little or no effect on strain 51B.

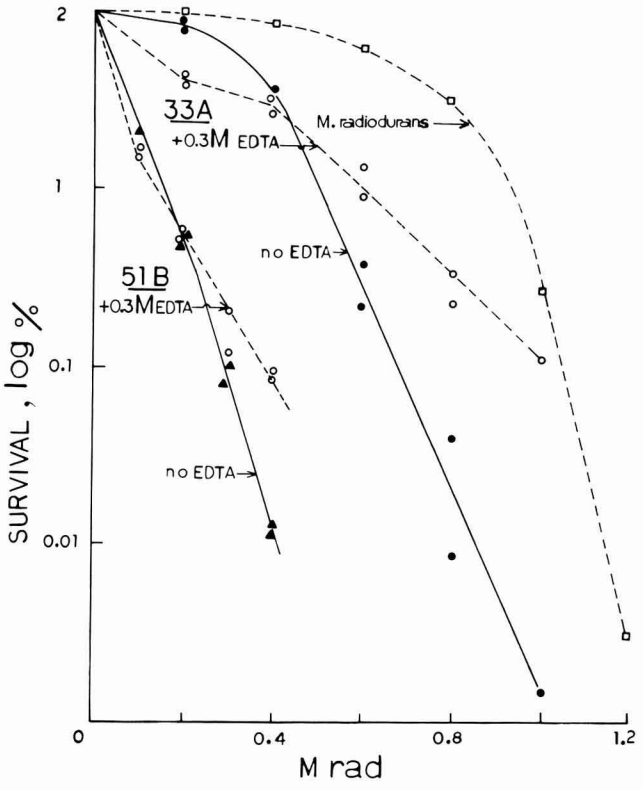

FIG. 6. EFFECT OF EDTA DURING IRRADIATION ON THE SURVIVAL OF *C. BOTULINUM* SPORES

Symbols: strain 33A without EDTA (●); strain 33A irradiated in the presence of 0.02 M EDTA (O); strain 51B without EDTA (▲); strain 51B irradiated in the presence of 0.02 M EDTA (○). Dashed line shows gamma survival curve of *Micrococcus radiodurans* (□ ——— □)

Interestingly, at radiation doses in excess of the shoulder, i.e. in the exponential decline portion (E) of the survival curve, EDTA provided radiation protection to both 33A and 51B (Fig. 6). This dual role of EDTA seems to be related to its two distinct properties, namely: (1) the ability to chelate and withdraw Mg^{++} which is essential for DNA ligase; this results in sensitization of spores particularly in the shoulder, and (2) the ability to serve as radioprotector when present during irradiation (Haugaard *et al.* 1957), probably via the hydrocarbon portion of the molecule as in the case of ethanol (Friedman and Grecz 1974). Therefore, in subsequent experiments, EDTA was added after irradiation to eliminate its radioprotective effect during irradiation.

Survival of 33A was reduced by ca 45% when the spores were irradiated while frozen at −75C (dry ice) and, after irradiation, exposed to 0.03 M EDTA for 1 h to inhibit repair in the dormant spores (Table 6).

Table 6. Effect of EDTA treatment on survival of spores of *Clostridium botulinum* 33A[a]

Addition of EDTA During Thawing of Sample[b]	Addition of EDTA to Recovery Medium (M)	Viable Colony Counts in	
		Unirradiated (control)	Irradiated (300 krads)
None	None	98	36
0.03 M, 1h, 30 °C	None	100	19

[a]Tubes contained 0.1-ml samples of a suspension of *C. botulinum* 33A at a concentration of 2×10^8 viable spores per ml. These were bubbled with nitrogen gas for 5 min, frozen in dry ice while under nigrogen gas, and irradiated to 300 krads while at −75 °C. This low temperature was applied to stop enzyme action in the spore. Controls were similarly treated but not irradiated.

[b]0.1 ml of water or 0.1 ml of 0.06 M sodium-EDTA solution (to give a final concentration of 0.03 M EDTA in the sample) was added to the spore sample after irradiation while still at −75 °C. The tubes were vigorously agitated on the Vortex mixer at room temperature so that the spores would come in contact with the EDTA as soon as possible. After samples reached room temperature, they were allowed to incubate at 30 °C for 1 h to permit any interaction with EDTA that may take place. Next the samples were diluted 10^{-6} to give approximately 100 colonies in the unirradiated control tubes. Colony counts in oval test tubes were set up in Wynne's agar (Wynne *et al.* 1955)

At 0.03M, EDTA showed maximum effect in depressing viable counts by ca 80-90%; at lower or higher EDTA concentrations the effect was distinctly diminished. A similar depression of recovery of irradiated spores was observed with other metal sequestering agents such as EGTA, citrate, and polyphosphate (Fig. 7). It should be noted in Fig. 7 that EDTA-inhibition of spore recovery was considerably higher than that by EGTA. This corresponds with the more than 1000 fold higher binding constant for Mg^{2+} exhibited by EDTA (Sillen and Martell 1964).

Figure 8 confirms that the inhibition of EDTA was a specific one due

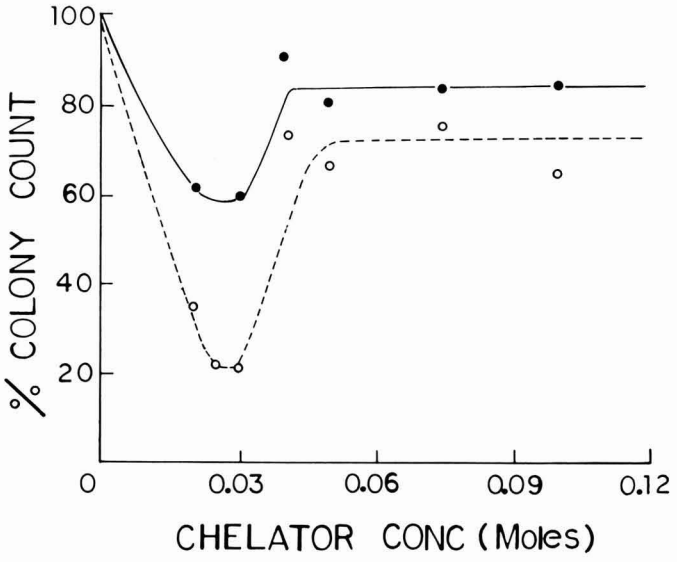

FIG. 7. EFFECT OF EDTA AND EGTA ON RECOVERY OF SPORES OF *C. BOTULINUM* 33A IRRADIATED WITH 0.3 MRAD

These chelators inhibit recovery by withdrawing Mg^{2+} from spores; the stability constants (K) for binding Mg^{2+} are for EDTA log K = 8.7; for EGTA log K = 5.2 (Sillen and Martel 1964). This experiment was conducted in an essentially identical manner as that described in Table 6, footnotes *a* and *b*. ○ EDTA, ● EGTA.

to sequestering of Mg^{++} since addition of 0.04 M Mg^{++} after dilution of the EDTA-treated spores restored viability essentially to that of untreated (control) spores. While the same concentration of Mn^{++}, Ca^{++}, or Zn^{++} had little or no effect. Spores irradiated under oxic conditions were not affected by EDTA or Mg^{++}. Prolonged storage of irradiated spores in presence of EDTA resulted in irrevocable loss of the spore's ability to recover. As shown in Fig. 8, after 24 h in 0.03M EDTA the irradiated spores lost their capacity to repair radiation damage in response to added Mg^{++}. This suggests that the initial DNA-lesions in spores may have changed to a non-repairable form, perhaps to 5'OH-3'OH gaps as suggested in the discussion. The enzymatic nature of direct repair of spore recovery in 33A was further supported by the observation that the process was temperature dependent; within 1h, at 0°C, 15°C to 60°C, and 75°C repair was completed to 55%, 95—100% and 2%, respectively.

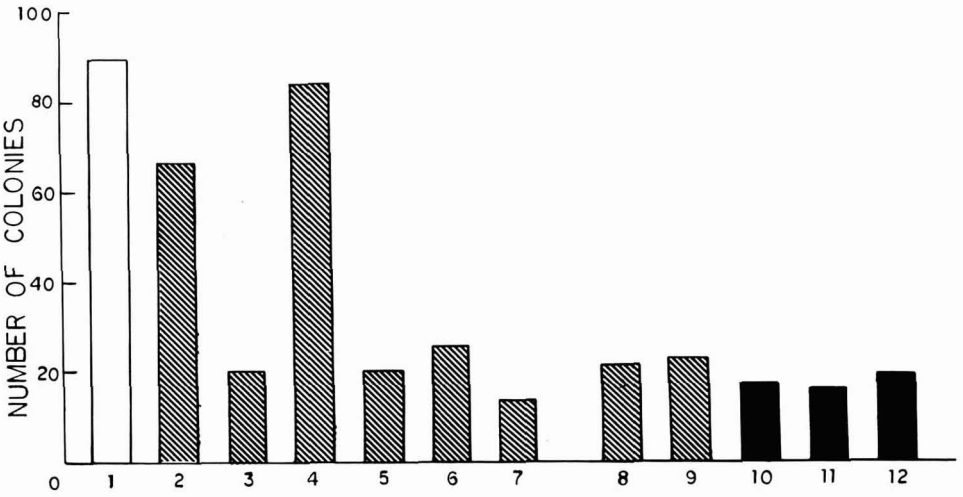

FIG. 8. ROLE OF CATIONS IN REVERSING RECOVERY OF EDTA-TREATED SPORES OF *C. BOTULINUM* 33A

The experiment was conducted in an essentially identical manner as that described in Table 6, footnotes a and b. Bar shows viable counts after irradiation at −75 °C with 0.3 Mrad:

□ 1. Unirradiated control (frozen only)

□ 2. to 9. Spores irradiated in N_2 atmosphere with 0.3 Mrad ^{137}Cs γ rays

2. No EDTA added during thawing of sample (irradiated control)
3. 0.03 M EDTA (final concentration) added prior to thawing and incubated 1 h at 30 °C prior to plating
4. 0.03 M EDTA (final concentration) added to sample prior to thawing, incubated for 1 h at 30 °C, diluted 10^{-6}, 0.04 M Mg++ added, incubated for 1 h at 30 °C, plated in oval tubes in Wynne's Agar (Wynne *et al.* 1955).

5—7. Identical to sample 4 except that Ca^{2+}, Zn^{2+}, and Mn^{2+} (in that order) were added instead of Mg^{2+}

8. As sample 3 except that sample was treated with 0.03 M EDTA for 24 h at 30C
9. As sample 4, except that sample was treated with 0.03 M EDTA for 24 h at 30 °C then diluted 10^{-6} and subjected to 0.04 Mg^{++} for 1 h in an attempt to reverse the EDTA affect. As seen Mg^{2+} was no longer able to reverse the EDTA effect.

■ 10—12. Spores irradiated in O_2-atmosphere with 0.3 Mrad ^{137}Cs γ rays

10. No EDTA added (irradiated control)
11. EDTA added as in sample 3
12. EDTA and Mg^{2+} added as in sample 4. Spores irradiated in presence of O_2 are not affected by either EDTA or by Mg++ suggesting that DNA SSB are not rejoined after irradiation in presence of O_2.

DISCUSSION

DNA Lesions

In order to more fully understand the mechanism of DNA repair (or the failure of repair) it is important to know the nature of DNA lesions induced by radiation. Many types of damage may contribute to cell death, however, much of the available information to date is concerned with single and double strand breaks in the DNA, their yield, rejoining and biological significance (Johansen 1975). Studies of *in vitro* model systems (Ward 1975) and *in vivo* cell DNA (Johansen 1975) reveal a multitude of mechanisms of possible DNA strand breakage resulting in different DNA strand termini. Depending on the nature of the DNA strand breaks different repair mechanisms must be employed by the cell. In this sense, the nature of the strand breaks undoubtedly plays a most crucial biological role in cell recovery from initial radiation injury.

Single strand breaks with $5'PO_4$, 3'OH and 5'OH termini were detected in irradiated *E. coli* cells by alkaline phosphatase, polynucleotide kinase, DNA ligase and DNA polymerase reactions. The total quantity of 5' termini corresponded to those detected by the method of alkaline sucrose gradient sedimentation (Gaziev *et al.* 1974).

Rejoining of broken strands as soon as 30—40 s. after irradiation (Johansen 1975) is an inherent complication in determination of the initial yield of radiation induced strand breaks *in vivo*, because many breaks may have rejoined by polynucleotide ligase, prior to analysis. On the other hand, "ligase-specific" DNA breaks may be transformed into unbindable form during a short period of post-irradiation incubation. The concept of DNA breaks in the cell exposed to ionizing radiations is summarized in Fig. 9. In applying the available information to bacterial spores the special dehydrated and calcified cytoplasmic environment of the spore must be taken into account.

Direct Rejoining of DNA Strand Breaks in Vegetative Cells

Radiation resistant strains of vegetative bacteria are able to repair initial DNA breaks if irradiated under anoxic conditions, but not breaks formed in presence of oxygen (Altman *et al.* 1970). DNA breaks are rejoined to 40—70% during anoxic irradiation at 0 °C (Dean *et al.* 1969; Gaziev *et al.* 1974). Generally, SSB are not lethal to radiation-resistant bacteria; however, SSB may become lethal to radiation-sensitive bacteria lacking appropriate repair systems (Weiss and Richardson 1967) or bacteria irradiated in the presence of SSB repair inhibitors, such as oxygen or ethylenediaminetetraacetic acid (EDTA) (Dean *et al.* 1969).

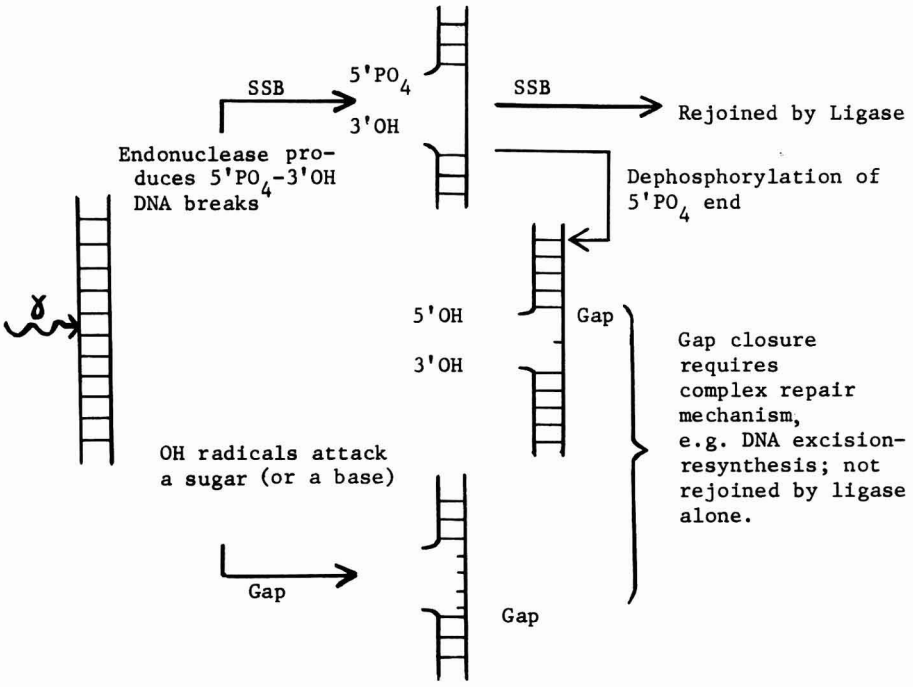

FIG. 9. DNA LESIONS AND THEIR REPAIR

Single strand breaks (SSB) and single strand gaps produced *in vivo* as the result of exposure to ionizing radiation.

In vitro, EDTA has been shown to completely inhibit ligase-adenylate intermediate formation which is essential for DNA ligase repair activity (Little *et al.* 1967).

Rejoining of SSB in resistant vegetative bacteria is accomplished in the absence of active metabolism, namely, in stationary cells under conditions of metabolic cell rest in absence of added nutrients and in the presence of chloramphenicol and temperature-time conditions precluding significant metabolic activity, i.e. 0°C, 40 min. It has been suggested that this type of SSB repair may involve one single enzyme, DNA ligase (Dean *et al.* 1969; Dean and Pauling 1970; Little *et al.* 1967; Mizutani *et al.* 1971).

Genetic studies of Seeberg and Rupp (1975) particularly with *E. coli* ligts 7, a temperature sensitive ligase deficient mutant, support the unique role of polynucleotide ligase in rapid rejoining of DNA strand breaks. Furthermore, their results with *E. coli* mutants supported the idea that

endonuclease is responsible for DNA breaks which in turn can be rejoined by ligase. These authors claim that a similar DNA breaking-rejoining cycle can be simulated *in vitro* by the use of purified enzymes.

Ligases have been isolated and characterized in several laboratories since 1967 (Lehman 1974). Transformation of $5'PO_4$ termini into ligase-adenylate-complex at the moment of irradiation contributes to quick rejoining of the DNA strand breaks (Gaziev *et al.* 1974).

Direct Rejoining of DNA in Spores

Resting spores in the state of cryptobiosis have an extremely low rate of respiration (Church and Halvorson 1957; Crook 1952) and therefore, enzymatic processes such as DNA repair have been generally thought to be either absent or of minor significance.

Recently, DNA repair has been demonstrated in *Bacillus subtilis* during early stages of germination, before any enzyme synthesis (Tanooka and Terano 1970; Terano *et al.* 1969, 1971). It was concluded, therefore, that repair enzymes are present in the dormant spore but that they are restrained by the special conditions of spore cryptobiosis.

Subsequently the existence of direct rejoining of DNA breaks in dormant non-germinated spores of the radiation resistant strain *C. botulinum* 33A has been reported (Durban *et al.* 1974) indicating that even under the conditions of cryptobiosis this type of rejoining of DNA breaks does occur in radiation resistant spores.

Since spore cytoplasm is essentially dehydrated (Carstensen *et al.* 1971) and highly calcified (Grecz *et al.* 1972), it is clear that DNA excision-resynthesis repair is impossible prior to germination. Therefore, it is attractive and logical to speculate that it is the direct rejoining of $5'PO_4$-3'OH DNA breaks which is the primary mechanism for DNA maintenance during irradiation or shortly thereafter. The inhibition of recovery by EDTA (Fig. 6-7) and oxygen (Fig. 8) seems to relate direct DNA rejoining processes to the shoulder in the radiation survival curve. Furthermore, since L is generally correlated with over-all radiation resistance of *C. botulinum* strains, the ability for direct rejoining of DNA breaks may be the major explanation of differences in radiation resistance observed in various strains of this organism.

Analogously, the extremely high capacity for direct rejoining of strand breaks in *Micrococcus radiodurans* (Dridger and Greyston 1971) may be related to the extremely large shoulder (L) in its radiation survival curve (Fig. 6).

If one assumes that initially as many SSB were produced by 0.3 Mrad in radiation resistant strain 33A as in the radiation sensitive strain 51B,

i.e. at least 28 SSB per chromosome than the 3 SSB detected by our sedimentation studies (Table 5) suggest that 33A spores had the capacity to rejoin at least 50—90% of the initial DNA strand breaks. This may account for the very large shoulder, L = 0.35—0.67 Mrad and extremely high extrapolation number, N = 39—90 (Table 3) in the radiation survival curve of this strain.

The evidence to date that DNA-ligase is responsible for rejoining DNA SSB in radiation resistant spores of 33A can be summarized as follows: (1) Repair was strongly temperature dependent characteristic of enzyme involvement; (2) The specific Mg^{++} requirement at a narrow optimum concentration is characteristic of DNA-ligase; (3) Inability to repair radiation damage produced under oxic conditions is characteristic of DNA-ligase; (4) DNA repair under essentially non-physiological conditions is characteristic for DNA-ligase, i.e. at 0 °C, in media lacking a source of energy, at pH 2, in non-germinated spores suspended in buffer.

Failure of Cell Recovery

The practically important aim in radiation sterilization is not spore recovery discussed above but rather the opposite, i.e. the failure to recover. At the molecular level the reason(s) for failure to recover are not yet clear. One of the possible complications of the DNA rejoining process resulting in spore death may be indicated in Fig. 8 where it is shown that irradiated sproes of 33A when stored in presence of ligase inhibitor (EDTA) for 2 to 24 h, gradually lose their ability to recover even after addition of Mg^{++} (Wiatr 1974). Recognition of the nature of this irreversible change may be of great practical importance to food engineering. Some indication may perhaps be found in the observation (Gaziev *et al.* 1974) that in *E. coli* 5'OH termini in DNA strand breaks accumulate in addition to 5'PO_4 and 3'OH termini. The appearance of 5'OH ends may be explained as the result of dephosphorylation of 5'PO_4 ends as suggested in Fig. 9. Phosphatases necessary for the postulated dephosphorylation step are ubiguitous and hydrolyze many different esters of phosphoric acid (Lehninger 1970). Some have strong preference for the 5'PO_4 in duplex DNA (Kornberg 1974). Since 5'OH-3'OH strand break constitutes a gap in the DNA strand, it cannot be directly rejoined by polynucleotide ligase, and would require a more complicated DNA repair operation such as e.g. excision resynthesis repair which involves several DNA repair enzymes in addition to ligase. Excision repair would be expected to occur only under fully metabolic conditions in the cell cytoplasm. In bacterial spores, this would only be possible after germination and resumption of active metabolism.

It seems safe to conclude that with respect to DNA maintenance the radiation damaged spore requires both mechanisms of DNA repair, namely (1) the immediate rejoining of strand breaks by ligase (in the shoulder of the radiation survival curve) and (2) the subsequent DNA resuscitation notably by excision-resynthesis and possibly other repair mechanisms triggered after spore germination and resumption of cell metabolism.

Relevance to Food Sterilization

Modern food process engineering should be based on a clear understanding of basic cell mechanisms responsible for cell disfunction and loss of viability. This is particularly important with respect to bacterial spores which constitute some of the most resistant and most hazardous agents of food spoilage.

The knowledge of DNA breakage and DNA resuscitation is of particular importance and may have much broader significance than realized so far. Recently several lethal agents, in addition to radiation have been related to DNA breakage and DNA repair including heat (Woodcock and Grigg 1972), freezing (Alur and Grecz 1975; Long and Grecz unpublished), hydrogen peroxide (Ananthaswamy *et al.* 1976), and ozone (Poliquin *et al.* 1976). However, with few exceptions only vegetative cells or extracted enzymes have been studied, and the results are generally not applicable to spores in view of the special cytoplasmic and structural spore features. It is clear then, that basic research in the area of molecular biology of bacterial spores has not kept pace with the swift progress in related areas of molecular cell biology.

The existing emphasis on pragmatic solutions and practical data that dominates the field of sterilization technology is, in the modern view, essentially simplistic, wasteful and out of date.

In spite of the immense practical importance of basic aspects of spore injury and spore recovery to food poisoning, and to medical and pharmaceutical sterilization, basic research in this area has not so far received the emphasis and financial support that it truly deserves. It is not surprising therefore, that the current understanding of molecular biology of spores with respect to DNA injury and recovery such as described in this paper is still fragmentary and substantially rudimentary.

Consistent with current progress in molecular cell biology it is essential that stronger support be provided for basic research into the molecular basis of spore resistance, spore injury and recovery. At the same time, however, close liason must be maintained between the basic

and applied laboratory in order to provide a sound basis for meaningful progress in all areas of sterilization technology.

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ERRATA

The paper, MICROSTRUCTURE OF FREEZE DRIED EMULSIONS, published in Volume 2, Number 3, of the *Journal of Food Processing and Preservation* contained errors. The corrections are given below.

Page 207. The last line in the second paragraph should read:
. . . in a Sorvall Omnimixer for 10 min after adding the oil phase.

Page 208. The 12th line of the first complete paragraph should read:
. . . 50 °C. The total fat content of a freeze dried sample is also obtained . . .

Page 211. The second line from the bottom of the page should read:
. . . (i.e. as free fat).

Page 212. The first sentence in the second paragraph under the heading Carboxymethyl Cellulose (CMC) should read:
Figure 2 shows an OM of the CMC emulsion.

Page 215. The first text line under Fig. 5 should read:
. . . droplets since surface energy effects favor spreading of the oil. Ex-. . .

Page 219. The caption below Fig. 11 should read:
OPTICAL MICROSCOPE VIEW OF SAME FILLED AS IN FIG. 10 (400X)

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39 U. S. C. 3626 provides in pertinent part: "No person who would have been entitled to mail matter under former section 4359 of this title shall mail such matter at the rates provided under this subsection unless he files annually with the Postal Service a written request for permission to mail matter at such rates."

In accordance with the provisions of this statute, I hereby request permission to mail the publication named in Item 1 at the phased postage rates presently authorized by 39 U. S. C. 3626.

SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER
John J. O'Neil, President

PS Form 3526 Mar. 1977 *(Page 1)*

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Prof. T. P. Labuza, Editor, Journal of Food Processing and Preservation, University of Minnesota, Department of Food Science and Nutrition, Saint Paul, Minnesota 55108 USA

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CONTENTS