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Isolation and determination of nutritional factors in food and fodder yeast

Robert Lee Smith
Iowa State College

NOTE TO USERS

ISOLATION AND DETERMINATION OF NUTRITIONAL FACTORS IN FOOD AND FODDER YEAST

by

Robert Lee Smith

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

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I. INTRODUCTION

Food and fodder yeasts represent a valuable source of many of the chemical compounds found in the diets of well-satisfied men and animals. They are especially valuable since methods are available for growing them on a wide variety of carbohydrate-containing materials commonly classified as waste or surplus, i.e., waste sulfite liquor, molasses or hydrolyzed grain, wood or cellulosic wastes.

Yeast will be most valuable in the future of nutrition as a food supplement rather than as a sole or primary dietary ingredient. It has been limited in food usage because of undesirable taste characteristics. The maximum threshold of dried yeast addition to bland foods is considered to be between 1 and 3 per cent. This varies with the type of yeast and the type of food. Since yeast as a commodity has not met with universal acceptance, one of the purposes of this investigation has been to determine whether the protein constituent can be isolated and made acceptable.

Foods may be broken down into five categories of constituents, namely, proteins, lipids, carbohydrates, vitamins and minerals. In addition to known representatives of these five categories, yeasts contain many, as yet, unidentified factors.

On a daily consumption basis, high-quality proteins for the human diet have been the most difficult of the food constituents to produce, protect and deliver. Food and fodder yeasts have a high protein

content. They can convert inorganic nitrogen into protein more efficiently than our conventional plant and animal food protein sources. However, the low taste threshold has definitely limited the use of yeast to a vitamin supplement rather than a protein supplement in the human diet.

Protein molecules are very large; consequently, they should have very little taste. Most of the taste in yeast, therefore, can be attributed to the small molecules accompanying the protein. By removing these small molecules it should be possible to produce a protein bland enough to be used as a protein supplement. This necessitates the breakage or alteration of the cell wall in order to release the cell contents for further processing. Hydrolysis, autolysis and osmolysis have been the more commonly accepted commercial methods of breaking yeast cells when the preservation of vitamins has been the primary concern. A more rapid and less expensive method is desirable for the production of protein concentrates.

An important purpose of this investigation was to determine whether it was feasible to use an exploding technique to break yeast cell walls. This process involves the rapid release of internal steam pressure from the cells. This exploded yeast material and other forms of yeast were processed in order to find a method for producing a bland protein concentrate. Another phase of the investigation involved the development of methods for isolating cellulolytic organisms and using them to help in isolating an unknown factor(s) in yeast which promotes cellulolytic action.

II. REVIEW OF THE LITERATURE

A. Introduction

The literature pertaining to yeasts is very large. Chemical Abstracts lists approximately 1500 separate papers relating to yeasts in the years 1947 through 1953. Many of these papers, as well as the practical experience dating back to antiquity, have dealt with the use of yeast as a food or fodder supplement. In recent years, however, an extensive bibliography has been developed pertaining to the various chemical constituents of the yeast cell.

This literature survey was designed to review some of the past work pertinent to this investigation. First, it was necessary to find the yeast cell-wall composition, in order to facilitate the understanding and development of the physical or chemical attack necessary to break the cell wall barrier and release the cell contents for further processing. Second, a survey of the various laboratory and commercial methods for breaking yeast cells has been made. Third, a description of yeast proteins has been included and an outline correlating information about yeast proteins for human food on a safety, quality, quantity unit-cost basis. Last, a table of yeast cell constituents has been compiled, in order to facilitate the investigation of the unknown factors and to aid in recognizing the chemical constituents which must be removed in order to produce a bland yeast protein.

B. Yeast Cell Wall Rupture

According to Skinner, Emmons and Tsuchiya (229), the growth form of yeasts is predominantly unicellular. The structure of the single cell may be represented as a protoplast contained within a cell wall. The cell wall is a tough membrane which is very elastic and resistant to abrasion. There is another membrane, called the cytoplasmic membrane, which lines the inside of the cell wall (18, 12, 70, 89). This can be clearly demonstrated by examining isolated cell walls with an electron microscope.

Inside the cytoplasmic membrane, according to Skinner, Emmons and Tsuchiya (229), Knaysi (125) and Wallerstein (268), is the cytoplasm. This is a complex proteinaceous material of a rather homogeneous composition. Included in the matrix of the cytoplasm is a relatively small nucleus and numerous granules and vacuoles which contain reserve foodstuffs and waste products. Frequently volutin inclusions, which are composed largely of free ribonucleic acid, are found. Lipoid inclusions, by-products of growth, are also found in the cytoplasm of some yeasts.

In order to make it possible to use yeast as an industrial or laboratory raw material for the production of proteins, fats, carbohydrates, vitamins or biological intermediates, it is, first, necessary to break the yeast cell wall or change its permeability in order to release the cell contents for further processing. Many different ways have been devised for breaking yeast cells in the

laboratory; however, due to economic considerations not all of them are feasible for industrial application. Since this is a rather important unit operation, a better knowledge of the composition of the cell wall should make it easier to devise and improve methods for breaking yeast cells.

According to Neuberg (172), the cell wall comprises 2.4 per cent of the total dry weight of the yeast. It is a tough material which is predominantly composed of polysaccharides. Table I gives the average composition of yeast cell walls as calculated from the data of several workers (52, 105, 144, 146, 147, 171, 174, 176, 177, 184, 210, 223, 231, 256, 258). The composition of the various constituents are based on the per cent by weight of the dry cell wall. The average nitrogen content of the cell wall, if multiplied by 6.25 would indicate that the crude protein content is approximately 13 per cent. It is known that the nitrogen in the cell wall is not all in the form of protein. However, paper chromatography techniques have indicated the presence of glutamic acid, aspartic acid, serine, glycine, threonine, alanine and faint traces of histidine, lucine and other amino acids.

Many of the methods used for breaking bacterial cells may be applied to yeast. A review of some of the bacterial methods was made by Werkman and Wood (274) in 1941.

1. Autolysis

According to Skinner, Emmons and Tsuchiya (229), yeast cells which have been killed by a treatment which does not destroy their enzyme

Table I

Composition of yeast cell walls

Constituent		Per cent of dry cell wall
Total carbohydrate		68.0
Chitin		7.0
Glucan		29.0
Mannan		31.0
Glycogen (associated with cell wall but not a structural part)		-
Nitrogen		2.1
Lipid (mainly neutral fat)		
Nitrogen	0.1% of lipid	
Phosphorous	0.5% of lipid	
Ash		3.0
Total phosphorous		0.3

systems will undergo autolysis. As the name implies, autolysis is a process brought about by the yeast's own enzyme system and consists in the successive degradation of the various cell constituents. Included in this degradation is the rupture of the cell wall which consequently releases the cytoplasmic material.

Since autolysis is an enzymatic process, it seems reasonable to assume that the most rapid rate of autolysis would occur at optimum conditions for enzymatic activity. These optimum conditions will, of necessity, be an average because, according to Dernby (49), not all of the enzymes present respond to the same optimum conditions. It has been established that the rate of autolysis varies with the pH, temperature and additives which affect the media in which the cells are dispersed.

Farrer (65, 66) has shown that bakers' yeast in water has an optimum temperature for autolysis of 50° to 55° C. at pH 5. At this optimum pH and temperature, it required only 80 minutes to 3 hours for the complete liberation of thiamine, riboflavin and nicotinic acid. On the other hand, pantothenic acid was not completely released even after 45 hours. There is a lag period (65, 66) after mixing pressed yeast and water before autolysis begins. This lag period is followed by a relatively rapid release of the vitamins and nitrogen compounds. The length of the lag period can be noticeably shortened by cold storage of the yeast cells prior to autolysis.

Commercial patents by Kahn and coworkers (115, 116, 117) reveal that at temperatures of 36° to 44° C. brewers' yeast requires from

3 to 20 days for autolysis. This seems to be an unreasonably long autolysis time for that range of temperatures in view of the fact that Payen (185) was able to get optimum nitrogen release in 48 hours with pressed yeast and in 72 hours with dry bakers' yeast at 6° to 8° C. Windisch and coworkers (282) have shown that 79 per cent of the albumin (based on nitrogen analysis) is hydrolyzed to peptones in 3 hours at an optimum temperature of 55° C. with a pH of 5.0. They have also shown that 89 per cent of the albumin is hydrolyzed in 6 hours at a temperature of 52° C. at a pH of 5.0.

Many different antiseptic agents have been used to kill yeast cells preparatory to autolysis. It is most desirable for an antiseptic to kill the yeast cell without altering the enzymes or the conditions established for optimum enzyme activity.

Payen (185) used ether as an antiseptic while the Societe des Produits Alimentaires, Azotes (233) in their 1931 German patent recommended 5 to 10 per cent ethyl alcohol. Havassart (168, 169) has shown that 2, 4 or 5 per cent boric acid, saturated benzoic acid solution, salicylic acid, 5 or 10 per cent alcohol, mustard oil and saturated or one-half saturated toluene were all effective antiseptics which did not impair enzymatic activity. He (168, 169) has shown that autolysis is diminished by 0.2 per cent sodium carbonate and completely inhibited by 0.4 per cent sodium carbonate, while hydrochloric acid decreases autolysis. The latter is probably a pH effect accompanied by a denaturation of the enzyme proteins.

Kahn and coworkers (115, 117) used salt and an extractive from brewers' yeast as antiseptics for autolysis. In the case of salt, plasmolysis occurred simultaneously with the autolysis. Pavcek (182) reported that yeast autolysis in Germany has been accomplished as follows: Compressed yeast was mixed with 2 per cent salt and agitated for 2 to 3 hours. The liquified mass was heated at 50° C. for 24 hours, then raised to 80° C. and held at this temperature for 12 hours. The product was clarified by centrifugation and concentrated in vacuo to produce yeast extract.

Lacroix (132) has prepared a salt free yeast extract by autolysis using 3 per cent trichloroethylene as an antiseptic and to increase the cell wall permeability. Apparently only a few minutes are necessary before the yeast mixture liquifies under these conditions. Griessbach and Ambros (84) used ethyl acetate as an antiseptic. Their United States patent claimed that this autolysis increases the commercial yield of some compounds, such as ergosterol, over non-autolysis techniques.

2. Osmolysis

Osmolysis is a process which causes the disruption of cell functions by a difference in osmotic pressure between the cell and the medium. This action may be divided into two categories, both of which are useful for recovering cell constituents. They are: 1. Plasmolysis (125), which occurs when the osmotic pressure of the medium is greater than that in the cell. The protoplasm loses its water, and there is a general shriveling of the cell as the cytoplasm shrinks away from the

cell wall. 2. Plasmoptysis, which occurs when the osmotic pressure of the medium is lower than that in the cell. Water tends to enter and cause the cell to swell. If the osmotic pressure differential is great enough the cell wall will burst.

Lux (152) was granted a British patent to plasmoptysize or physiologically disrupt yeast cells by treating them with distilled water. Aries (9) in his United States patent also mentioned the use of distilled water for plasmoptysis of yeast cells.

Aries (8) revealed the plasmolyzing procedure used by the yeast plant at Regensburg, Germany. Here a 12 per cent yeast suspension is washed twice with 2 times its volume of water and then one volume of 10 per cent sodium chloride is added. The suspension is then heated at 60° to 70° C. for 4 hours to plasmolyse the yeast. According to Aries (8) and Loesecke (149) a 20 to 25 per cent salt solution is commonly used for plasmolysis.

Astrup and Øhlenschläger (11) used chloroform, benzene, sodium chloride, and 33 per cent w/v urea solution as plasmolyzing agents. A more effective way of plasmolyzing yeast cells was developed by Pirie (189) who used a mixture consisting of 20 parts by volume of sulfuric acid, 100 parts ethanol and 80 parts ether in plasmolyzing yeast cells for the production of glutathione.

Kunitz and McDonald (131) used toluene to plasmolyze yeast cells prior to the purification of hexokinase. About 25 pounds of yeast were thoroughly mixed with 6 liters of warm toluene and heated on a

warm water bath to 37° C. It was held at this temperature for 2 or 3 hours, then cooled, diluted and let stand for 18 hours at 5° C. This procedure combines plasmolysis and autolysis.

Weizmann (272) was granted a British patent in 1940 which described how to plasmolyze yeasts by the use of sugar and organic acids found in fruit juices. For example, plasmolysis was completed in 3 minutes when 2.5 grams of concentrated pineapple juice (containing 52 per cent sugar and 5.2 per cent acid, calculated as citric) were added to 25 grams of fresh bakers' yeast.

3. Hydrolysis

Yeast cells may be ruptured by the hydrolytic action of acids or bases. Unfortunately, the hydrolysis is not confined to the structural materials of the cell wall but also affects the cell constituents as they are released. This irreversibly alters some of the cell constituents making it impossible to isolate them in their natural state. The necessity of neutralizing the acid or base is usually a detrimental feature of hydrolysis. This not only increases the labor and expense of the operation but also results in an undesirably high salt concentration.

Many variations of reagents, temperature and equipment have been used for yeast hydrolysis. Loesecke (149) in 1946 stated in his review that hydrochloric acid is often the reagent of choice for yeast hydrolysis. One method of hydrolysis employing 5 per cent hydrochloric acid and 35° to 80° C. required 4 days for completion. Higher

temperatures require a shorter hydrolysis time but also result in greater destruction of cell constituents.

In order to by-pass the alkaline debittering of brewers' yeast when making yeast extracts, Nilson (175) kept the taste producing hop resins insoluble while he hydrolyzed the cells with acid. The hydrolysis was performed at 77° C. by heating the yeast cells with dilute (1 part concentrated acid to 400 parts water) hydrochloric acid for about one-half hour. The cell residue was filtered off and the acid neutralized with sodium carbonate while the temperature was maintained at 77° to 93° C. During the neutralization some albuminoid material precipitates.

Johnson and Harkins (111) succeeded in releasing a protein free nucleic acid by a process which began with the alkaline hydrolysis of yeast cells with 4.5 to 5 per cent sodium hydroxide at 0° C.

4. Mechanical cell rupture

Yeast cells are small, and their cell walls are tough and elastic. As a consequence, the mechanical methods used for breaking animal and plant tissue cell walls do not generally prove practical for breaking yeast cells commercially. In the laboratory, however, where power and efficiency are relatively unimportant factors, many mechanical methods have been tried for breaking yeast cells. Attrition by use of various abrasives has probably received the most attention.

The Potter-Elvehjem homogenizer (192) works very well for breaking the cells of liver, brain and kidney tissues. This apparatus consists

of a test tube containing the tissue in which a close fitting glass pestle is rotated at about 100 r.p.m. The pestle has small glass beads fused to the end to facilitate disintegration of the tissues. It has been reported (192) that for some models there was 0.23 mm. clearance between the test tube wall and the pestle. This would obviously limit its effectiveness on yeast cells with average diameters of 10 μ .

Dockstader and Halvorson (56) in 1950 reported the use of a modified Potter-Elvehjem homogenizer for breaking bacterial cells. No radical design changes were made but closer tolerances were obtained between the tube and pestle. They (56) have compared the advantages and disadvantages of the Potter-Elvehjem homogenizer with the Wood-Werkman mill (260) and mortar and pestle methods for grinding bacteria.

Kalnitsky, Utter and Werkman (118) in 1945 reported the development of a cell grinding mill. Bacterial cells in the form of a paste, as harvested by Sharples supercentrifuge at 35,000 r.p.m., were mixed with ground glass. The paste of bacteria and ground glass was then forced between the interfaces of two, closely fitting, ground-glass concentric cones. The inner cone was rotated at about 150 r.p.m. to provide the attrition necessary to rupture the cells. The inner cone acted as a reservoir for crushed ice to keep the apparatus cool. The crushed cells were caught in a suitable receptacle and then the ground glass was removed from the soluble cell material by centrifugation. The proportions of wet bacterial paste to glass were in the range of 1:2 to 1:8. The glass particles averaged 2 μ in size.

Muys (166) in 1949 modified the bacterial grinding mill principle of Kalnitsky, Utter and Werkman (118) to include automatic feeding of the bacterial suspension. The contact area of the ground glass surfaces was reduced to a minimum. This allowed increased pressure and decreased the tolerances which made the ground glass abrasive unnecessary. Other features were: enclosure of the apparatus to eliminate exposure to air, shorter grinding time, and self operation.

Many different materials have been used as abrasives for the mechanical comminution of yeast cells. Alexander's American patent (190) called for the use of one part by weight of diatomaceous silica per two parts of yeast on a dry cell basis. The mixed yeast cake is pulverized and dried. The soluble yeast components are then extracted by use of 0.1 M. solution of phosphate buffer of pH 7.2.

Lux (152, 1953) and Kronig (129) mixed quartz sand with yeast and broke the cells by pressing the mixture through closely disposed rolls or by spraying it under high pressure against smooth or roughened surfaces. Ground glass (145, 277), sand (11) or kieselguhr (92) mixed with yeast and triturated with a mortar and pestle or ball mill has been used frequently in the laboratory. In these methods a part of the protein and other cell constituents may be denatured, altered or adsorbed to the abrasive particles. Lindquist (146) ground a mixture of yeast cells and dry ice with a mortar and pestle to break the cells.

A Mickle disintegrator (177) has been used to break yeast cells in the laboratory. The cells are agitated with glass beads in this

apparatus. Curran and Evans (46) in 1942 published their observations on the effect of agitating bacterial spores with various abrasives. The number of viable spores remaining after agitation for 5 hours was used as the criterion for comparing the effectiveness of several abrasives. Glass beads proved to be the most lethal. Alundum, emery, sand and boron carbide were the least effective.

Lux (153) obtained a British patent in 1930 which claimed yeast cells could be broken with a colloid mill. He describes this mill in detail in the patent. Torrington (255) in 1940, however, pointed out that colloid mills have been relatively inefficient. This is due to the toughness and elasticity of the yeast cell walls, as well as the fact that the mechanical heat developed destroys all but the heat stable vitamins.

Lux (152, 153) and Kronig (129) report the use of disintegrators, gear pumps, spark discharges and radiation with Rontgen rays to break yeast cells. No data are given on the effectiveness of these methods.

Hughes (106) in 1951 reported the successful disruption of yeast and bacterial cells by application of a sudden shearing pressure to cells mixed with abrasive or ice crystals. The apparatus consisted of two polished, stainless steel blocks which were held together by bolts. A piston well was drilled in the clamped blocks centering at the interface. On the inner face of one of the blocks a reservoir channel was cut. The only connection between the reservoir and the piston well was the very narrow slit or interface between the two polished blocks. The

pistons were machined to fit the piston well within tolerances of 0.002 to 0.003 inch.

The procedure followed was to mix the cell suspension with ground glass (average section 5.0 μ^2) or freeze the block and cell suspension to -20° C. with dry ice. The material to be disintegrated was then placed in the piston well and the piston placed into position. With all bolts tightened and the piston hand-tight against the material, the piston was hit with a fly press. This caused the material to be forced along the interface from the piston well into the reservoir. The fly press exerted 12 to 15 tons per square inch pressure on the piston. This resulted in nearly 100 per cent cell breakage when the abrasive was used and 80 to 90 per cent breakage at low temperatures where ice crystals acted as the abrasive. Alumina, zircon, emery and carborundum proved to be as effective as ground glass when they were used as abrasives.

Booth and Green (28) have developed a wet crushing mill for breaking yeast cells. The crushing is accomplished by placing a yeast suspension in a roller bearing race and rotating the bearing at about 600 r.p.m. The tolerances between the rollers and the race are reduced to zero by pressing the cone shaped rollers into a similarly tapered race. Any heat is dissipated by circulating the yeast cream through coils cooled by ice water. Approximately 30 minutes are required to reduce the cell count of a 40 ml. sample to 10 per cent.

5. Effect of thermal variation on cell rupture

Changes in thermal conditions have been studied rather extensively for their effects on cell viability and on cell wall rupture. Extremes of temperature have been studied, as well as rapid variations of temperature.

Freezing changes the permeability of yeast cell walls and allows some of the cell contents to be released. Ulrich (259) claims that the greatest destruction of the cells is brought about by quick freezing. He has suggested that holding frozen yeast cells at a temperature just below freezing, rather than at extremely low temperatures, is most effective for cell disruption during storage. This, he explains, is due to continuous ice crystal transformations in this temperature range. Saccharomyces cerevisiae seems to be surprisingly resistant to extremely low temperatures. For example, Wallerstein (268) cites one experiment where the cells remained viable even after 20 minutes' exposure to -150° C.

A review of the effects of extreme cold on bacterial cells was given by Weiser and Osterud (271) in 1945. They reported that repeated freezing and thawing are more destructive to bacteria than a single freezing. They also reported that freezing is more destructive than supercooling and that in some cases extremely low freezing temperatures are more destructive than less severe temperatures. On the other hand, cases have been found where there was little difference in the lethal effect at temperatures ranging from 0.0 to -195° C.

The greatest mortality from freezing occurs when bacteria are suspended in water. Other media such as sugar, glycerol, milk, peptone, colloids and the like, protect the bacteria from the external effects of growing ice crystals. From the data a controversy has arisen concerning the importance of the crushing effect of external ice in the medium on the mortality of the bacteria.

Weiser and Osterud (271) explained that bacteria die slowly at low temperatures, primarily, because of starvation or destructive metabolism. The destruction, however, is continuous and is a function of time and temperature. They (271) believed it unlikely that intracellular ice contributes much to the lethal effect.

Lund and Halvorson (151) have shown that there is a continuous destruction of yeast cells in a frozen medium and that rapid freezing causes greater injury to yeasts than slow freezing. Freezing and thawing a suspension of aerobically grown yeast cells causes less destruction than for anaerobically grown cells. The rate of thawing has a profound effect on the viability, as well as on the enzymatic activity of the cell (151, 271). The slower the rate of thawing, the greater the lethal effect. The composition of the medium profoundly influences this. For example, mineral and organic acids both enhance the lethal effect of freezing.

Haurowitz (92) and Kench (121) stated that repeated freezing and thawing is one of the simplest and best methods for destruction of cellular membranes. Haurowitz (92) expressed the opinion that part of the action is due to osmotic pressure changes which occur when pure ice

crystals freeze and concentrate the cell salts in the residual unfrozen cell liquor and that part is mechanical due to rupturing of the cell wall by ice crystals.

Rückforth (212) found that he could rupture yeast cell walls by freezing the yeast at -12° to -15° C. This was followed by rapid heating to a temperature of 42° C. It was necessary to crush the frozen yeast mass before heating. The heat was applied by hot water or by passing the yeast between heated rollers. He stated that freezing alone does not rupture the cells or greatly alter the viability. Black (23) used liquid nitrogen to freeze bakers' yeast and then extracted it with warm 0.3 M dibasic potassium phosphate to initiate the purification and concentration of yeast aldehydedehydrogenase.

High temperatures have been effective in rupturing yeast membranes. A considerable amount of effort has been expended in finding out the effects of heat on cell constituents. Unfortunately, heat has proved to be detrimental to many of them. Kressel (128) in 1902 claimed that heat could be used to break yeast cells and hydrolyze the protein of their cytoplasm to peptones. He employed direct steam injection at 180° to 200° C. for one-half hour.

Overbeck (179) obtained an American patent in 1899, claiming the rupture of yeast cells by heating. His method was to place pressed yeast in boiling water and boil until the yeast mass was liquefied and the cells broken. No heating time was specified for breaking the cells. Experimental work substantiating the fact that heat ruptures cell

membranes was done by Salton and Horne (219). They used the electron microscope to determine the extent of rupturing of bacterial cells at various temperatures.

Balls (15) prepared a food product from yeast by using heat to break the yeast cells. He used 50 g. of compressed yeast mixed with 350 ml. of water and heated the resulting suspension in an autoclave for 30 to 60 minutes at 150° C. This treatment resulted in disruption of the cells and liquefaction of the yeast slurry. A heating time of 1 hour at 115° C. was insufficient to remove the odor and flavor which the higher temperature accomplished.

Wallerstein (268) mentioned in his review in 1940 that, generally, moist yeast cells are completely killed at 50° to 60° C., whereas, dry yeasts require appreciably higher temperatures for destruction. The influence of heat upon the yeast cell constituents will vary directly with the degree of heat and the time of exposure. Many of the cell constituents are heat labile and will be destroyed. The protein of the cell is subject to denaturation with subsequent destruction of enzymatic properties. With due consideration for these facts, Pollatschek (190) has secured a Swiss patent which concerns the solubilization of part of the yeast proteins by heating yeast to boiling at 20 mm. pressure. After filtering off the cell residues the solution is vacuum evaporated. The resulting extract is claimed to be rich in protein and the various vitamins of the B-complex. The temperature used to vacuum evaporate the water from yeast extract is given by Pavcek (182) as 50° to 58° C. The time, including the time of autolysis

at the same temperature, exceeded 30 hours. In spite of this, 2 to 3 mg. of thiamine and 200 to 350 units of riboflavin were present per 100 g. of dry yeast.

Schormüller (225) has shown that heating yeasts 2 hours at 200° C. resulted in a 34 per cent loss of histidine, whereas, moderate heating or storage of yeast did not destroy the histidine content appreciably. Loesecke (149) in his review stated that some of the B vitamins are lost during drum-drying operations. However, brewers' yeast is commercially dried on drum driers heated with 80 p.s.i. steam pressure. According to analyses given by MacDonough and Hoffenreffer (156) the yeast still retains a high vitamin content. Sometimes yeast is enriched with vitamin solutions just prior to drying. This indicates that the destruction of the added vitamins is economically insignificant. Thiamine, riboflavin and niacin are the usual vitamins used for enriching. Aries (9) has found that over-heating during the yeast drying operation deleteriously affects the quality of yeast proteins used for adhesives.

Folic acid hexaglutamate is conjugated with yeast protein by an extremely dissociable salt-like linkage, according to Allfrey and King (6). The addition of ethanol, acetone or similar solvents will result in almost complete cleavage of the folic acid-protein bond. When folic acid hexaglutamate and its associated protein are heated for 1 minute at 80° C., or for 5 minutes at 60° C., substantially all of the folic acid hexaglutamate is released from the associated protein. Dialysis also causes the folic acid hexaglutamate to cleave from the protein and appear in the diffusate. This action is pH dependent.

6. Cell rupture by pressure variation

Many different procedures have been tried to test the effect of static pressures and variations of pressure on yeast cells. Wallerstein (268) stated that yeasts can survive under quite high static pressures. In testing to determine whether pressure was injurious to yeast vitality, one culture of Saccharomyces cerevisiae grew after being subjected to 4,000 p.s.i. for 10 minutes. However, at this pressure the smaller cells seemed to be destroyed selectively. Yeasts require higher pressures for destruction than bacteria.

Torrington (255), in his review, mentioned that various combinations of pressure and vacuum have been used to break yeast cells. In one process, liquid containing yeast was subjected to high pressure and then discharged through an orifice into a vacuum. He explained that this process did not operate efficiently because the liquid within the cells and the surrounding medium was practically incompressible. Therefore, the main action from discharge through the orifice was mechanical atomization.

Milner and coworkers (165) used 20,000 p.s.i. pressure to force yeast cells through the orifice of a needle valve. About 20 per cent of the cells were broken by this action. The apparatus consisted of a steel block with a hole drilled in it. One end of the hole was necked down and tapped to receive a needle valve. A piston with close tolerances fitted the other end of the hole. The yeast suspension was placed in the block, and pressure was applied to the piston by a hydraulic press.

Fraser (73) was able to break bacterial cells by rapidly releasing the cells under high pressure. He introduced the cell suspension into a sterile tank. Nitrous oxide or carbon dioxide was then forced into the tank to the desired pressure. After allowing 3 minutes for the pressure in the cells to equilibrate to the tank pressure, the tank was inverted and the cells rapidly released into a beaker. At pressures of 500 to 900 p.s.i. up to 96 per cent loss of viability was achieved. Nitrous oxide was ineffective at 250 p.s.i., but satisfactory at 500 to 800 p.s.i. Argon and nitrogen require a minimum of 900 p.s.i. to be effective. It was thought that their low solubility might account for this. Best results were achieved with bacteria which were harvested just prior to the end of logarithmic growth.

It has been recognized that yeast cells are small and elastic and that they tend to distort under applied external pressure without appreciable shearing action. As a consequence, cell disruption should best be accomplished by creating a high internal cell pressure. There should exist a critical internal-external cell pressure differential sufficient to rupture the cell wall. The disintegration of cellular materials by the rapid release of vapor pressure (exploding process) is not new. There have been several methods used for building up the internal cell pressure. This is demonstrated by the following examples.

The process of puffing wheat and other cereal grains for use in breakfast cereals is a well-known industrial process of our era. There has not, however, been any extensive literature dealing with the application of this process to microorganisms. Anderson (7) obtained

the United States patent in 1902 which covers the exploding or puffing of starch and cereal grains. The patent explains this phenomenon, essentially, as follows: The grain or starchy material should be heated under any suitable pressure and following this, while the material is still hot, the pressure should be suddenly reduced. The pressure should be reduced sufficiently below the point at which the cellular liquid boils to cause the liquid to gasify. This gasification expands or explodes the cereal grain or starch granules. The exploding effect is caused by the sudden rupture of the cell wall material and subsequent release of the internal pressure. This pressure differential is due to the restraint of the gasses within the cell by the relatively impermeable cell wall during the time the external pressure is released. In this process the temperature and pressure are dependent variables.

Anderson's (7) process for exploding grain required an autoclave temperature of 125° to 300° C. and a heating time of 10 to 45 minutes. He found that it was best not to maintain the grain at maximum temperature for extended periods. Excess heat resulted in charring of the starchy material. There were variations in effect and taste even at the temperature and time limits given.

Rabinowitsch (196) obtained a United States patent in 1931 claiming the disintegration of cereal grain cells by rapid release of pressure. His process employed temperatures of 30° to 35° C. and the pressure was built up to 3.5 atmospheres by fermentation with grain enzymes. When the pressure in the fermentation autoclave was released the aleurone cells of the grain were completely disintegrated. He also claimed that mold spores were disintegrated during this process.

Mason (160) obtained a United States patent in 1926 claiming to disintegrate wood by exploding it. His process required a preliminary tempering of the wood with steam at pressures not exceeding 350 p.s.i. for a fractional part of a minute. Immediately following the tempering process, a gas such as air, oxygen or nitrogen was introduced into the reactor. This increased the pressure to 700 to 1,000 p.s.i. The gas was used to avoid the charring caused by the high temperatures of steam at that pressure. Less than a minute was required to equilibrate the pressure inside the wood chips with that of the ractor. The outlet was then opened and the material exploded into the atmosphere.

It was found necessary to maintain the high pressure in the reactor for the duration of the explosion of the material. As a consequence, the minimum outlet orifice allowable was one tenth of the diameter of the reactor. Otherwise, constriction of the orifice by initially exploded material caused a back pressure. This resulted in less efficient fibration of the last part of the wood exploded.

A method of exploding yeast cells was patented in 1940 by Torrington (255). His process began by allowing yeast to ferment. This was done in an effort to increase the carbon dioxide content of the cells. The fermenting yeast slurry was then pumped through a dispersion pump at 100 p.s.i. and 32° F. Carbon dioxide was then introduced into the slurry and the dispersion pump used to mix it intimately. He (254) found that increased efficiency was obtained by allowing the carbon dioxide to equilibrate into the cells for 48 to 72 hours. The pressure on the slurry was next raised until it was between 1,000 and

3,000 p.s.i. This material then passed through a heat exchanger which raised the temperature to 49° C. This was done to convert all carbonic acid to carbon dioxide. The mixture was then discharged through a spray head into a vacuum tank.

Torrington (255) claimed that the cells were broken for three reasons. 1. The mechanical atomization from the high pressure discharge through the spray head, as well as the release of the carbon dioxide dispersed in the medium contributed to the cell breakage. 2. The carbon dioxide in the cell, from fermentation and penetration of the high pressure carbon dioxide surrounding the cells, explosively expands and breaks the cell wall. 3. The action is facilitated by the weakening action of adding nutrients to dormant cells to make them ferment.

Yeasts have a tendency to absorb the vitamins from their media and incorporate them into their cell structure during brewing. As a result, modern methods of filtering out the brewers' yeast tends to produce a vitamin free beer. With this in mind, Ditto and Torrington (55) have used the carbon dioxide exploding technique to remove the vitamins from the yeast so that they can be used to vitamin-enrich beer.

7. Miscellaneous processes for cell rupture

Several devices are available commercially for disintegrating cells by ultra-sonic vibrations. They tend to be rather expensive and inefficient. This may make them impractical for large scale commercial operations. Stumpf, Green and Smith (242) in 1945 outlined briefly the procedure and apparatus necessary to disintegrate bacterial cells by

exposure to an ultra-sonic field. A quartz crystal oscillator with a frequency range from 200 to 1,000 kilocycles was used. Yeast cells were ruptured by Meyerhof and Ohlmeyer (164) with a sonic vibrator. They vibrated a yeast suspension (6 g. in 20 ml.) for 75 minutes at 9 kilocycles per second to release active adenosine triphosphatase. Other methods of cell rupture had inactivated this enzyme.

Csonka (145) has shown that ether changes the permeability of the yeast cell wall. The action is two-fold. First, it kills the yeast cell. Second, it dissolves out the lipid substances and makes the cell wall more permeable. This effect is demonstrated by the increase in protein extraction by various solvents.

Any treatment which makes the yeast cell wall more permeable will allow certain of the cell contents to be extracted. The amount and kind of cell constituents extracted depends upon the solvent and the degree of permeability change. Neuberg (172) reported that only the cell membrane remains undissolved when yeast is treated with alcohol, ether and acetone and then with very dilute alkali.

Drying changes the permeability of yeast cell walls. Aries (9) took out a patent covering the use of dried yeast cells as a raw material for the production of proteins. The fat is first removed from the dried yeast with petroleum ether and then the protein is extracted with 0.05 N. sodium hydroxide. Thorsell and Myrbäck (253) pointed out that water may be used to extract codehydrogenase I and codecarboxylase from dried yeast cells. Koyama (127) used water with a small amount of ammonia in it to extract adhesive proteins from dried yeast.

Combinations of two or more of the above-mentioned methods are frequently used for rupturing yeast cells. Lindquist (145, 147) freeze-dried yeast and ground the material in a stainless steel ball mill for 15 minutes to 24 hours. The mill was partially evacuated and cooled with ice. This was apparently a two-stage process. During the first stage, the friction of the dried cells caused an increase in the cell wall permeability, and during the second stage the cell structure was disintegrated.

Yeast cells may be ruptured by enzymatic attack. An analogous situation occurs when bacteria are disintegrated by enzymes in tears, saliva, trypsin and the like.

C. Food Protein from Yeast

From a nutritional standpoint yeasts contain varying amounts of the food constituents, namely, proteins, lipids, carbohydrates, vitamins, minerals and unknown factors. The economic survival and expansion of the yeast industry depends upon its ability to maintain a competitive advantage in the production, protection and delivery of one or more of these food constituents.

Competitive advantage alone does not insure economic survival. This can be insured only by acceptance of the product by the buying public. This acceptance can be won honestly only by comparing yeast constituents with all of its competitors in the field on the basis of all aspects of safety, quality, quantity and unit cost.

The advent of low-cost synthetic vitamins and other chemicals has relegated yeast to the role of emphasizing those constituents which are of such a complex nature that their commercial synthesis is not yet feasible. It follows from this that any unknown factors which when isolated prove to be readily synthesizable, will follow the same economic pattern as the vitamins.

Minerals for the human diet are available from many sources and do not constitute a bottleneck of production. It is possible that research may prove that certain minerals are most beneficial when eaten in organic form. On the other hand, it has been found that ferrous sulfate and iodized salt are very satisfactory sources of iron, sulfur, sodium, chlorine and iodine. If this proves to be the case for other minerals, it will lessen the economic importance of yeast as a dietary mineral source.

Proteins, fats and carbohydrates are required in much larger amounts in the daily diet than are vitamins and minerals. Consequently they represent a much larger market potential than do the vitamins and minerals. With the exception of a few localities, there is a greater paucity of food proteins, especially high-quality proteins, in the world than of carbohydrates and fats. Food protein molecules are complex, and it is difficult to foresee their commercial synthesis in the near future. These facts coupled with the fact that yeasts can very efficiently convert inorganic nitrogen into protein nitrogen using a variety of low-grade carbohydrate energy sources, should be ample justification for further research on yeast proteins.

1. Nature of yeast proteins

First, it is necessary to define yeast proteins. After this has been accomplished, it will be possible to correlate the information on food yeast protein on the basis of safety, quality, quantity and unit cost. This correlation of data will reveal the deficiencies in knowledge which need to be filled in by research. Diaz (50) has made a very useful annotated bibliography for food yeasts.

The quantities of at least some of the chemical and food constituents in yeasts will vary with the species and the medium in which they are grown. Bearing these inconsistencies in mind, the analysis ranges for the gross constituents of bakers', brewers' and torula food yeast are given in Table II. These data are average values calculated from the data of several workers (2, 8, 30, 36, 54, 81, 144, 145, 146, 171, 256, 257, 258, 269, 278).

Crude protein accounts for 25 to 65 per cent of the dry weight of yeast. It will be noticed, from the data of Table II, that a significant amount of the nitrogen is in the nucleic acid and lipid fraction of the yeast. The true proteins of yeast, according to Lindquist (145), Virtanen (265) and Wiley (280), are primarily enzyme proteins since yeasts are not considered to have reserve proteins. Csonka (45) believes that many of these proteins are conjugated with nucleic acids to form nucleoproteins.

Lindquist (145) was able to separate yeast proteins into five fractions, each having a nearly uniform sedimentation constant. These

Table II

Food constituents of yeast[a]

Constituent	Per cent constituent	Per cent nitrogen in yeast attributed to constituent
Amino acids, peptides and proteins (% N = 16)	20.0 - 52.0	3.2 - 8.3
Nucleic acids (% N = 14.2)	- 8.0	- 1.2
Fats and lipids (% N = 4.5)	1.6 - 6.0	0.07 - 0.3
Carbohydrates (by difference)	20.0 - 47.0	- 0.5
Vitamins	very small %	- -
Minerals	7.0 - 9.0	

[a]Based on dry weight of yeast.

five fractions, however, were not electrophoretically uniform, i.e., fraction one contained at least seven main components. Stern, Schein and Wallerstein (235) outline the essential steps in salt fractionation of yeast proteins.

Carter (36) reported that attempts to isolate and characterize yeast proteins have met with little success. This has been due to the fact that the cell proteins are altered during isolation. Proteolytic activity during autolysis and chemical disruption during most of the cell rupturing procedures causes alteration of the physical and chemical nature of the proteins. He (36) reviewed the fact that water extraction of defatted, ruptured yeast cells removes a thermolabile and a thermostable protein. Subsequently a globulin can be extracted with dilute salt solution and other proteinaceous material can be removed by alkali extraction.

Alexander (5) claimed that yeast proteins are normally found in their native state as corpuscular molecules. It has been commonly accepted that their biological activity is dependent upon the maintenance of the native physical and chemical state. However, treatment of yeasts with drastic chemical substances, such as acid or base, tends to change the corpuscular protein irreversibly to a fibrous type of molecule which has quite different properties.

Kunitz and McDonald (131) have isolated several crystalline proteins from yeast. Various concentrations of ammonium sulfate were used to fractionally precipitate the proteins. One of the proteins

formed fine needle-like crystals; another formed prisms. A yellow protein which they isolated was classified by Ball (14) as having at least two prosthetic groups. One of these prosthetic groups is a flavin; the other is unidentified. The above proteins were isolated during the purification of hexokinase. This is an albumin type protein with a molecular weight of about 96,000. Berger and coworkers (20) also prepared hexokinase from yeast using ethanol as a selective precipitant for the protein fractions instead of ammonium sulfate.

Levy and Coon (140) autolyzed yeast under acetone. Alcohol-ether (1:1) and 5 per cent trichloracetic acid were the reagents which they used to precipitate the protein. They were able to remove the nucleic acids from these proteins by suspending them overnight in a 10 per cent sodium chloride solution on the steam bath. Gilvarg and Bloch (77) isolated 2 g. of protein from 10 g. of dried yeast by autolyzing with ether, extracting with 0.2 per cent sodium hydroxide and precipitating the protein with 5 per cent trichloracetic acid. Aries (9) was able to obtain 8 to 16 per cent yields of yeast protein by extracting defatted dry yeast cells with 0.05 N. sodium hydroxide. He precipitated the protein by agitating it at the isoelectric point of pH 3.14 to 3.45. He was also able to precipitate the yeast proteins by using ethanol or other water soluble alcohols.

Csonka (45) in reviewing yeast protein literature stated that 10 per cent ammonium carbonate solution has been used to extract as much as 65.7 per cent of the total yeast nitrogen. A globulin and an albumin protein were extracted from yeast using this procedure. He (45)

also reported the isolation of a paranucleoprotein which is but slightly soluble in 10 per cent sodium chloride and is not coagulated by heat. An albumin coagulable in water at 40° C. was also isolated.

Ether extraction of yeast cells followed by treatment with water or 10 per cent sodium chloride has been used to extract a water-soluble, heat-coagulable protein. This protein may also be precipitated by 65 per cent saturated ammonium sulfate. Csonka (45) found that the 10 per cent salt soluble protein was not a true globulin. It did not separate by water dilution or by dialysis. It has an isoelectric point of pH 4.7. It was found that by pretreating yeast cells with ether, up to 9.9 per cent of the total yeast nitrogen was heat coagulable and water extractable, and up to 8.3 per cent was heat coagulable and salt extractable. By treating yeast cells with ether and then successively extracting the cells with protein solvents the following quantities of coagulable protein were extracted: water, 8.24 g. or 13.4 per cent of total nitrogen; 10 per cent sodium chloride, 2.08 g. or 3.6 per cent of total nitrogen; 0.2 per cent sodium hydroxide 5.38 g. or 9.5 per cent of the total nitrogen. The total coagulable protein extracted was 15.7 g. representing 26.5 per cent of the total nitrogen.

The foregoing information on the isolation and properties of specific yeast proteins was given in order to point out the complex nature of yeast proteins. For the most part, either yeast extracts or whole yeast has been the dietary source for yeast proteins. In neither case was it possible to get dietary information on any one specific yeast protein. Judging from the great variety of different enzymes

(see Table VI), each supposedly a separate protein entity, it may never be practical to obtain dietary data on anything but mixed yeast proteins. This does not need to detract from their dietary usefulness.

The value of a protein food for human and animal nutrition depends on many factors. 1. The primary concern, of course, is the question of safety. The protein must be produced in a safe form or by proper treatment be made safe or it is valueless as a foodstuff. 2. The quality of the protein is also of concern. Among the aspects of quality are included taste, texture, sight appeal, odor and all of the other qualities which are responsible for eating enjoyment. Last, but by no means least, the biological nutritive quality must be considered. 3. The quantity of the protein present in the food is also important, especially the amount which is digestible or available for nutritive purposes. 4. The unit-cost of the food-protein is also important. The cost should be computed with due regard to the value of all of the food constituents, namely, proteins, fats, carbohydrates, vitamins and minerals.

The economic future of yeast proteins depends upon their acceptance by the buying public. The best way for the public to recognize the value of yeast proteins is to compare all of the aspects of safety, quality, quantity and unit cost with the proteins from other sources.

2. Safety of yeast proteins

Loesecke (149) in reviewing the safety of yeast for human consumption mentioned that the published literature gives very little information

as to optimum amounts tolerated. He reported that as much as 255 g. of dried yeast per person per day has been ingested by pellagra patients without evidence of deleterious effects. On the other hand, some instances are recorded where as little as 3 g. of dried yeast or 12 to 24 g. (dry basis) of fresh yeast cakes caused distressing gastro-intestinal symptoms or laxative effect. This was not general as it occurred in only a few of the cases. Another instance was reported by Loesecke (149) in which 100 g. of yeast daily was tolerated. A very small percentage of those tested developed diarrhea, the others had no ill effects. The reason for this has not been established.

Wiley (279) mentioned that no evidence of toxicity has been found in either torula yeast or the spent sulfite liquor on which it is grown. The liver necrosis resulting from feeding torula yeast in excess of 20 per cent of the diet of rats is the result of a deficiency of Schwarz factor 3 (226). This may be easily corrected by adding the factor or by having a variety of foods in the diet.

Due to the relatively high nucleic acid content of yeasts, Loesecke (149) reported that ingestion of yeast caused an increase in uric acid and creatinine excretion. Blood pressure was also increased in some cases. This depended upon the dietary history of the subject and the amount of yeast consumed. The Council on Foods of the American Medical Association does not believe it probable that yeast in normal quantities will cause an increase in uric acid production sufficient to be harmful. Dirr (53) noted an increase of 1 mg. per cent in serum uric acid after the addition of 30 g. of dried torula yeast to the diet of

two humans. Bahl and Ahmad (13) found no significant increase in uric acid excretion during a 4-month test with 21 children fed 7 to 14 g. of dried yeast per day.

Yeast proteins are low in methionine (149); therefore, it is conceivable that, even in the presence of all supplementary growth factors, they would be detrimental due to this amino acid deficiency. This does not need to bar yeast as a dietary protein source since Chiao and Peterson (42) have indicated that the deficiencies of yeast protein may be corrected when yeast is fed in combination with other foods. It is also possible to enrich yeast proteins with synthetic methionine. They (42) pointed out that current literature indicates that about one-half of the protein ration can be replaced by yeast protein without affecting the growth rate of chicks.

Gray (81) pointed out that brewers' yeasts have long been used by the public with beneficial results as a supplement to provide protein and most of the B vitamins. On the other hand, experiments by Lindan and Work (143) showed that yeast does not contain all of the essentials necessary for a balanced diet. They have developed a diet using yeast as a sole source of protein which will induce liver necrosis in rats. The liver necrosis can be cured or avoided by including tocopherol, methionine and cystine in the diet in the proper amounts. Furthermore, Lindan and Work (142, 143) pointed out that brewers' yeast has less tendency to produce liver necrosis than bakers' yeast. A yeast diet deficient in tocopherol, tends to deplete the liver of glutathione. This has been shown by feeding low levels of any of several proteins and tocopherol.

It was shown by Hoff-Jørgensen (100) in 1947 that 8.2 per cent dried yeast in the diets of pigs caused them to develop rickets. This was shown not to be a toxic factor but was caused by a phytase inhibitor in the yeast. The symptoms were prevented by feeding vitamin D, increasing the calcium carbonate in the diet from 1.5 to 4 per cent or replacing yeast phosphate by sodium phosphate.

Bandt (16) has assumed that death of fish fed food yeast experimentally was due to a toxic effect. This toxicity, he claimed, is due to the detectable amounts of lead, copper and arsenic salts which are found in spent sulfite liquors on which the yeast was grown. Wiley (279) attributed this to impurities in the chemicals used for pulping the wood. In the United States, pure sulfur is used and toxic impurities are not present.

In summary it may be said that yeast proteins are non-toxic, providing that no toxic materials are present to be absorbed from the media upon which they are grown. It has been established that yeasts do not contain all of the constituents necessary for a well-balanced diet. Nevertheless, yeasts are valuable and may be safely consumed for the constituents which they do contain.

3. Quality of yeast proteins

Quality in nutrition embraces many tangible and intangible aspects. Foods are judged in commerce, at the present time, by the factors of taste, smell, texture, sight appeal and other inadequately defined qualities of good eating. As a consequence, yeasts have had a restricted

acceptance because as a commodity they possess certain characteristic flavors which make them undesirable to the buying public.

There is no doubt that food is the raw material which is produced, protected and delivered so that by its consumption the human race may be built and maintained. This raw material, food, is bought and sold, normally, by the above-mentioned quality standards. It seems reasonable to suspect that consciously or unconsciously people try to improve this raw material by making it meet ever higher quality specifications. This is done under the assumption that the food which meets the highest specifications, i.e., the best tasting, should be the best raw material. Thus, yeast with its relatively poor taste has been deemed a poor structural material. As a consequence, food yeasts have not had wide acceptance, in spite of the fact that they contain some of the valuable food constituents which are desperately needed by about two-thirds of the world's population.

In most instances, according to Brenner (31), addition of 1 per cent of yeast to a food does not appreciably affect the taste. On the other hand, a 3 per cent yeast level, even if not initially detectable, will develop a bitter yeasty taste on storage. This low taste threshold has limited the market potential of yeast for human consumption in the past. Some work has been done to try to improve the flavor of yeast products. The main goal in most of these experiments has been to try to extract only the bad taste, leaving the B vitamins intact.

Brasch (30) extracted dried Saccharomyces cerevisiae with Skellysolve B until the lipid content was about 0.033 per cent. The

extracted yeast was much lighter in color than the original. The results of a taste panel indicate that the taste of the extracted yeast, immediately after extraction, was preferable to the unextracted yeast. Prolonged storage caused some change in the extracted yeast which made it have an undesirable taste.

The yeast fat which Brasch (30) extracted had a strong yeast-like flavor and odor. Since the yeast was bland, this indicates that much of the taste can be concentrated in the fat or that the fat actually causes some of the bad taste in yeast.

Skellysolve B and petroleum ether were satisfactory solvents for yeast fat. Carbon tetrachloride, benzene, and diethyl ether were unsatisfactory because they were difficult to remove from the yeast even at temperatures up to 75° C. in a vacuum oven. Alcohols were not used for extracting yeast fats by Brasch (30) because they also extract the B vitamins. Brasch has also shown that yeast toasted at 115° C. in drying or solvent removal also has a bad organoleptic rating.

Laer (133) found that he could control the nucleoprotein content of yeast by varying the amount of nitrogen and phosphorous in the media. The carbohydrate energy source which he used was synthetic alcohol. Lowering the nucleic acid content of the yeast increased its desirability for food. Another way of controlling the nucleic acid content of yeast is suggested by the work of Gulland, Barker and Jordon (88). They found that when yeast nucleic acid is added to ovalbumin, there is clear electrophoretic evidence that a complex is formed at the isoelectric point. At a more acid pH there is a partial precipitation

of the complex, and at a more alkaline pH no complex forms and the two fractions migrate independently. This principle may be of some use in extracting nucleic acids from food yeast.

Loesecke (149) reported that a great deal of the spicy flavor and some of the vitamins are destroyed when yeast cells are hydrolyzed under pressure with hydrochloric acid. Thomsen (252) removed the yeast taste without impairing the digestibility or vitamin content. He mixed peptized yeast with about 10 times its weight of vegetable oil preheated to 100° C. The mixture was then dehydrated under vacuum and the oil separated and reused. Unless a solvent was used to remove the oil, it would tend to become rancid in a short while.

Maizel (159) was able to remove undesirable flavors from liquid yeast products by stirring the yeast with a melted, partial stearic acid ester of glycerol. Partial stearic acid esters of glycols, polyglycerols and sugar alcohols had the same effect. Upon solidifying, the esters absorbed the flavoring constituents. The vitamin contents were unaltered.

Holle (102) secured a patent on a process which produces taste-free yeast and yeast extract. His procedure requires that the yeast cells be plasmolyzed at 50° to 60° C. The plasmolyzed yeast is centrifuged while warm, and the solids washed twice with hot water. The residue is light gray in color and tasteless. It contains about 9 to 10 per cent nitrogen and 1.85 per cent nucleic acids.

Balls (15) removed the objectionable odor and taste from yeast by heating. He heated a 5 to 15 per cent yeast slurry until the cells were disrupted and the mass entirely liquified. The moisture was then evaporated to 30 to 45 per cent. The temperature and time of heating markedly affect the destruction of the yeast taste. He (15) found that it was necessary to heat the yeast at 150° C. for 30 to 60 minutes. Treatment for 1 hour at 115° C. was not sufficient to destroy the odor and flavor of the yeast. Heating for 90 minutes at 155° C. coagulated the yeast and made the final product immiscible in water.

One of the quality factors which has received too little emphasis by scientists and industrialists alike, is the protein biological nutritive quality. The fact that there is no protein biological scale established in commerce is ample evidence of this. The health and satisfaction which a well-balanced diet produces are the result of eating the proper amounts of all of the food constituents of the proper biological quality. The biological nutritive value of a food protein is due to several factors. Melnick (161) has summarized some of these as follows: 1. Of first concern is the availability of the amino acids, i.e., digestibility or accessibility to enzymatic degradation by the digestive tract. This may be directly related to the folding and sequence of amino acid residues in the protein molecule. 2. The amino acid content of the protein, especially the essential amino acids. 3. The ratio of the amino acids present.

Melnick (161) stated that it is quite well known that the intact, live yeast cell is very resistant to attack by the enzymes of the

digestive tract. Experiments with animals have shown that a large percentage of ingested live yeast cells appear in the feces unharmed. It, therefore, becomes apparent that some sort of processing must be used before the nutrients locked in the yeast cell can become available to the digestive system. Any process, such as drying, autolysis, osmolysis, hydrolysis, and the like, which will break or change the permeability of the yeast cell wall, will improve the availability of the yeast protein.

It has been found by Cannon (33), using the rat-repletion method, that the biological value of the protein in dried yeast was not quite, but almost, as good as that in hydrolyzed casein and lactalbumin. Yeast protein proved to be of higher biological value than the protein in soybeans, sunflower seeds, peanuts, navy beans and peas. Lindan and Work (1942, 1943) have shown that the cystine-methionine ratio in bakers' and brewers' yeast is very low compared to the ideal ratio for rats. Phenylalanine, lysine and leucine were also shown to be somewhat lower than that desired for rats. Table III shows the amino acid composition of yeasts. The quantities are calculated from the data of several workers (see individual amino acids in Table VI for references) for brewers', bakers' and torula yeasts.

It is important to determine the detrimental effects of processing on yeast proteins. Some indication of the effects can be obtained by comparison with the processing of other food proteins.

Heat is usually involved in one or more of the unit operations used in preparing yeast products. Melnick and Oser (162) in reviewing

Table III

Amino acid composition of yeasts

Amino acid	Per cent amino acid nitrogen of total nitrogen
Alanine	7.7 - 9.0
Arginine	5.0 - 10.0
Asparagine	0.4 - 0.5
Aspartic acid	7.0 - 9.0
Citrulline	- -
Cystine	0.2 - 0.68
Glutamic acid	5.3 - 12.0
Glutamine	0.3 -
Glycine	5.8 - 7.0
Histidine	3.6 - 7.0
Hydroxyglutamic acid	2.3 -
Isoleucine	2.6 - 3.7
Leucine	4.6 - 6.0
Lysine	5.9 - 15
Methionine	0.7 - 0.9
Norleucine	- -
Ornithine	1.0 -
Phenylalanine	1.0 - 3.0
Proline	2.6 - 3.8
Serine	3.0 - 6.2
Threonine	2.1 - 6.0
Tryptophan	0.7 - 1.4
Tyrosine	1.1 - 3.4
Valine	3.0 - 9.0

the effect of heat on milk proteins, edistin, blood globulin, meat, fish, coconut, soybeans and other legume proteins stated that in almost every case adverse effects have been reported. Excessive heat tends to impair the biological value. However, lysine supplementation has substantially corrected the nutritional deficiencies caused by the heat. This indicates that lysine is either destroyed or combined in such a manner as to be enzymatically inaccessible to the digestive system. Melnick and Oser (162) reported that in most cases the lysine becomes unavailable biologically without changing either the amino acid composition or the degree of protein digestibility. It is postulated that a new peptide linkage is formed as a result of heating. This involves the ϵ - amino group of lysine and a free carboxyl group of some dicarboxylic amino acid.

Schormüller (225) has shown that storage and moderately raised temperatures has no marked effect on the histidine content of yeasts. However, heating yeast for 2 hours at 200° C. resulted in a 34 per cent loss of histidine.

The effect of heat and pressure on yeast proteins may closely parallel the effects shown by Hall (90) for wheat gluten protein. He pointed out in his literature review that chemical hydrolysis of protein is customarily performed by heating with either acid or alkali. An example of this is the production of hydrolyzates commercially by heating the protein with 20 to 25 per cent hydrochloric acid for 5 to 16 hours. The hydrolysis time varies inversely with the temperature. It is customary to use temperatures varying from 108° to 125° C.

Hall (90) has shown that wheat gluten protein was 8.65 per cent hydrolyzed when heated with water for one-half hour at 225 p.s.i. and 190° C. Heating the same mixture at 235° C. and 1,000 p.s.i. resulted in 43.9 per cent hydrolysis. Hall (90) stated that excess acid, above that amount needed to form the acid salts of all the amino acids, must be present before a complete hydrolysis can be obtained. In order to get efficient hydrolysis with water as the catalyst, temperatures high enough to cause protein destruction are necessary. The preliminary experiments have shown that 75 to 80 p.s.i. is the maximum steam pressure which can be used without protein destruction. This is based on 30 minutes' heating time.

Melnick and Oser (162) and Sure (247), in reviewing the effect of heat processing on cereal proteins, stated that in feeding experiments on growing rats it was proved that gun explosion of cereals was detrimental to the protein. The ratio of gain in body weight to protein consumed was depressed two-thirds by the gun explosion of an oats-corn-rye mixture. Gun explosion of oats alone depressed the ratio by three-fourths. This effect is obviously a function of heat intensity since Melnick and Oser (162) have shown that when cereals are properly cooked no undesirable changes occur in the protein component, and their nutritive value appears to be equal to that of the unheated proteins.

Sure (247) has found that the proteins of some prepared wheat cereals have been injured in the processing to the extent that Wistar rats will not gain weight on them when used as a sole source of protein. He found that the addition of 0.4 per cent L-lysine hydrochloride

improved the protein efficiency and made it better than that of unheated whole wheat. Addition of 0.2 per cent DL-threonine, 0.1μg. of crystalline vitamin B_{12} and extract of condensed fish solubles improved the protein efficiency of the lysine-enriched protein.

Goyco and Asenjo (79) found that Torulopsis utilis No. 3 could be autoclaved while wet for 1 hour at 118° C. and still retain practically the same nutritional value as yeast not subjected to the heat treatment. They also found that 0.5 per cent methonine added to a diet containing yeast as the sole source of protein almost tripled its growth-promoting value.

4. Quantity of yeast proteins

The bulk of the nitrogen in yeast is incorporated in the protein and nucleic acids. This has been shown in Table II. Carter (36) stated that the total nitrogen of dried yeast varies from 6 to 11 per cent. Purine nitrogen comprises 10 per cent of the total nitrogen while pyrimidine nitrogen accounts for 4 per cent of the total nitrogen. Gulland (88) and Carter (36) pointed out that the nucleic acids of yeast are "statistical tetranucleotides." Each of the two pyrimidines (cytosine and uracil) contains 5 nitrogen atoms and each of the two purines (adenine and guanine) contains 10 nitrogen atoms. Thus the purines should contain twice as much nitrogen as the pyrimidines. However, the discrepancy in the division of the 14 per cent total nucleic acid nitrogen leaves the tetranucleotide theory in doubt.

A small part of the total yeast nitrogen is incorporated in the molecules of other constituents such as lecithin, cephalin, cytochrome,

vitamins, cozymase and others. Table VI lists other constituents. Dirr and Decker (54) and Carter (36), however, pointed out that 64 to 80 per cent of the nitrogen is incorporated in the amino acids, peptides or true proteins. According to the values in Table II, these compounds comprise 20 to 52 per cent of the total weight of dry yeast.

In yeast extracts where the enzymes are unharmed, the protein content is continuously being degraded by enzymatic hydrolysis. Lindquist (145) has shown this by nitrogen determination during dialysis experiments. A decrease in the protein content of 5 per cent per day was noted at 4° C. and a decrease of 10 per cent at 20° C. This was true of both, crude extracts and pure yeast protein fractions at a pH of 6.8. Enzyme inhibitors such as 0.1 per cent mercuric chloride inhibited the enzymatic hydrolysis. The data indicate enzymatic hydrolysis of proteins into amino acids and peptides and they do not imply that the nutritional qualities are affected.

Yeasts can convert inorganic nitrogen into protein more efficiently than can our conventional protein sources. Thaysen (251) asserted that approximately 80 per cent of the inorganic nitrogen supplied can be recovered in the yeast protein. He also pointed out that the rate of conversion of inorganic nitrogen to yeast protein is an important factor in food production. He stated his method of comparison as follows (251, p. 446):

> As for the rate of conversion of nitrogen to protein, let me mention that a fully-grown bullock weighing approximately half a ton, produces protein at the rate of 0.9 lb. per 24 hour day. If half a ton of soy beans--one of the most prolific protein-producing plants known--be planted and grown under most

> favourable conditions and the beans harvested, then their yield (calculated on a 24-hour output basis) would be 82 lb. But, if half a ton of Torulopsis (the type of yeast frequently used in protein synthesis) were grown under favourable and technically possible conditions, then 51 tons of protein would be synthesized in 24 hours.

Thaysen (251) claimed that the rulers of Germany in the second World War considered the technical production of yeast as important as the production of hydrocarbon fuels. The peak German production was about 15,000 tons per annum. They had plans, however, for the production of 130,000 tons per annum.

Yeasts can very efficiently utilize low-grade carbohydrates as energy sources for the production of protein (280, 204). For example, waste sulfite liquor, hydrolyzed wood and agricultural wastes, surplus cereal grains and molasses from various sources have all been used to produce yeast.

There is a tremendous potential of raw materials for yeast production in the world. In the United States we have barely tapped our resources for yeast production. The technical know-how for converting these resources into yeast protein is fairly well developed. They will be used when a profitable market has been developed for yeast constituents.

The brewing industry, according to Gray (81), could harvest as a by-product approximately 100,000 tons of residual yeast per year. In 1945, according to Brenner (31), 10,868,883 lbs. of brewers' yeast were dried of which 2,509,281 lbs. were debittered for human consumption. Another 5,700,000 lbs. of primary yeast were grown that year.

It has been estimated by Schleef (221) that the Pacific Northwest has enough spent sulfite liquor from paper pulp production to produce 46,784 tons of torula yeast per year. According to Wiley (280) and Inskeep, Wiley, Holderby and Hughes (107), the annual production of 2,500,000 tons of sulfite-pulp in the United States produces 500,000 tons of sugar which is wasted in the spent sulfite liquor. If used for yeast production, this sugar represents a potential of 250,000 tons of yeast or 125,000 tons of crude protein.

Hydrolyzed wood wastes can be utilized for the production of torula yeast. The estimated wood wastes for 1944 have been tabulated in Table IV. This represents the parts of the cut trees left in the woods, trees destroyed in logging, slash disposal and manufacturing wastes. These data were calculated from United States Department of Agriculture reports (262).

If we assume that the average weight of the wood wastes is 32 lbs./ $ft.^3$, then the wood wasted in 1944, according to Table IV, would have amounted to 9.65 million tons. If this wood had been properly hydrolyzed and the resulting sugars used to produce torula yeast, according to the methods outlined by Prescott and Dunn (193), about 2 million tons of yeast would have resulted. This represents a potential of 1 million tons of yeast protein.

There are many other raw materials which can be utilized for yeast production, i.e., beet and blackstrap molasses, citrus molasses, hydrolyzed agricultural wastes and the like. However, if we consider only the annual potential of the protein from the brewing industry

Table IV

Wood wastes in the U. S. in 1944

Product	Amount of waste million cubic feet
Lumber	4,567.3
Fuel wood	199.4
Pulp and paper	334.3
Hewed cross ties	254.2
Cooperage stock	215.9
Fence posts	21.6
Veneer	259.4
Round mine timbers	25.5
Shingles	45.2
Other	192.0
Total	6,024.8

(50,000 tons), the sulfite pulp industry (125,000 tons), and wood wastes (1 million tons), we have a protein potential in the United States of 1,175,000 tons per year.

The daily consumption of protein per person was about 90 g. in 1950 for the United States. This amounts to a total crude protein consumption of about 6 million tons per year. Thus, it can be seen that the potential from the above sources is only about one sixth of the annual protein consumed in the United States.

5. Unit cost of yeast protein

The cost of yeast protein as a food constituent has not been generally known in commerce. However, it compares very favorably with the cost of food constituent proteins from other food sources. Most protein foods are sold as commodities and very little effort has been made to compare the cost of producing, protecting and delivering foods competitively on the basis of their constituents. The field of constituent economics, especially protein economics, will have to be developed before the value of yeast proteins can be effectively presented to the public.

It is desirable for each of the food constituents to receive its proper competitive value in computing the value of yeast protein or any other food protein. Loesecke (149, p. 486) published Table V in June 1946 to compare the cost of yeast protein with other food protein sources. However, these data are computed with the total cost of the food being borne by the protein and without each of the constituents bearing its fair share. He recognized this. Nevertheless, Table V

Table V

Approximate cost of yeast protein in comparison with other proteinaceous foods[a, b, c]

Produce	Cost per lb.	Per cent protein content	Approximate cost of protein per lb.
Yeast, dry (5% moisture)	0.40	50.0	0.80
Soybean flour (low fat)	0.0482	40.0	0.120
Soybean flour (high fat)	0.0605	35.4	0.171
Whole milk (dry)	0.3313	26.9	1.23
Milk solids not fat (dry)	0.1625	35.4	0.458
Cheese	0.2325	24.4	0.953
Salmon, canned	0.3125	20.8	1.50
Eggs, dried	1.13	46.5	2.43
Beef, choice	0.22	21.3	1.03
Pork, loins	0.259	16.6	2.56

[a]Adapted from Loesecke (147, p. 486) from June 1946.

[b]Prices will vary with time and section of the country, but those given represent prevailing prices on the same market at time of compilation and are deemed satisfactory for comparable purposes.

[c]This tabulation does not take difference of protein quality into consideration.

offers a good opportunity to compare the costs of producing, protecting and delivering the protein from various foods. This principle of comparing the cost of food constituent proteins must be improved and made available to all who wish to use it, in order for yeast proteins to be recognized properly by the public.

D. Chemical Constituents of Yeast

Table VI is a tabulation of a considerable number of the chemical constituents found in yeast. This is not presented as a complete tabulation of constituents nor does it give a complete list of references for each compound. It is presented in the hope that it will be useful in the identification of unknown growth factors. It should also prove helpful in identifying the small taste producing molecules which are associated with yeast protein.

E. Unknown Factor Which Stimulates Cellulose Digestion by Rumen Organisms

Food and fodder yeasts contain an unknown factor(s) which stimulates cellulose digestion by rumen organisms. The literature and work concerning this factor(s) has been presented by Kitts (124), Quinn (195), Ruf (215) and Sijpestein (228). These workers have attributed the following characteristics to this factor(s):

1. It is soluble in water and in alcohol up to 70 per cent concentration.
2. It is heat stable.
3. It is adsorbed on Norite.

Table VI

Chemical constituents of yeast

Constituent	References
Acetoin (acetylmethylcarbinol)	4, 85, 114, 173, 231
Acetyl coenzyme A	154, 155, 231
Adenine	86, 136
Adenosine diphosphate (ADP)	245
Adenosine-5-phosphate	72
Adenosine triphosphate (ATP)	36, 136, 231
Adenylic acid (adenosine monophosphate)	36, 86, 136, 245
Alanine	2, 3, 77, 142
Albumin (protein)	45
Alpha-amino butyric acid	142
Alpha-ketoglutaric acid	71, 72
Ammonia	2, 3
Amylamine	108
Amylose	171
D-Arabinose	88
Arginine	2, 3, 27, 77, 142, 24, 25, 74, 107, 240
Aryl amine (non-acetylable)	37
Asparagine	142, 148, 182
Aspartic acid	2, 3, 77, 140, 142

Table VI (Continued)

Constituent	References
Beta-alanine	12, 142
Beta-carotene	149
Biacetyl	284
Biocytin	186, 283
Bios V	239
Biosterol	239
Biotin	12, 39, 107, 220, 270
Biotin complex	39
2,3-Butanediol (L, meso and D, L)	170
Carbamido acid	67
Carbohydrates, K_3, K_4 and K_5 (unknown structure)	144, 146
Carbon dioxide	64, 255, 257, 268, 278
Carotene (from R. gracilis)	285
Cebrosan (carbohydrate)	144, 146, 147
Cellulose (not a true cellulose)	171
Cephalin	30, 36
Cerevesin (protein)	268
Chitin	52, 105, 146, 210, 223
Chitosan sulfate	105
Choline	81, 149
Cisaconitic acid	71

Table VI (Continued)

Constituent	References
Citric acid	71, 72
Citrulline	208
Cocarboxylase	65, 71, 213, 231
Coenzyme A	17, 136, 231, 267
Coproporphyrin	121
Cryptosterol	30
Cysteic acid	142
Cystine	2, 3, 26, 27, 107, 24, 25, 42
Cytidylic acid	86
Cytochrome a, b, and c	36, 62, 245
Cytosine	86, 142
Diacetyl	4
Diadenosinetetraphosphate	245
Desoxyribonucleic acid	36, 136, 266
Desoxyribose	86, 87, 171, 266
Dextrin	158
Diphosphopyridine nucleotide (DPN) (coenzyme I) (cozymase)	12, 36, 136, 139, 231
Dulcitol	69, 171
Enzymes	
Aconitase	71
Adenosine diphosphatase	245

Table VI (Continued)

Constituent	References
Enzymes (continued)	
Adenosine triphosphatase	164, 245
Alcohol dehydrogenase	23, 200
Aldolase	245
Alpha-galactosidase	243
Alpha-glucomelezitase	181
Alpha-glucosidase	95
Amylodextrinase	243
Amylase	103
Arginine desiminase	208
Asparaginase	244
Beta-fructofuranosidase	95
Carbohydrases	95
Carboligase	245
Carboxylase	71, 138, 268
Catalase	211
Choline esterase	109
Cytochrome c peroxidase	245
Cytochrome c reductase	245
Desoxypentosenuclease	244
Diaphorases	281
Diastase	211

Table VI (Continued)

Constituent	References
Enzymes (continued)	
Emulsin (beta-glucosidase)	243
Enzyme c	123
Esterase	243
Flavokinase	120
Formic hydrogenlyase	141
Fructosanase	137
Fumarase	106
Galactozymase	248
Gluconokinase	216
Glutamic dehydrogenase	75
Glutaminase	244
D-Glyceraldehyde-3-phosphate dehydrogenase	201
Glycogenase	268
Haas enzyme	245
Hexokinase	126, 230, 250
Inulinase	103
Isocitric dehydrogenase	71, 72
Lactase	243
Lipase	211
Maltase	243

Table VI (Continued)

Constituent	References
Enzymes (continued)	
Old yellow enzyme	245
Peroxidase	211
Phosphatases	101
Phosphogluconic acid dehydrogenase	104
Phosphoglyceraldehyde dehydrogenase	245, 250
Phosphokinase	264
Phosphorylases	243, 244
Phosphotriose isomerase	245
Polygalacturonase	150
Proteases	245
Pyrophosphatase	93
Pyruvic carboxylase	245
Saccharase (invertase)	243, 268
Transaminase	75
Trehalase	243
Triose phosphate dehydrogenase	273
Tyrosine decarboxylase	264
Urease	103
Uridine nucleosidase	34
Yellow enzyme	245
Zymase (a mixture of enzymes)	243

Table VI (Continued)

Constituent	References
Ergosterol	30, 81, 84
Ethanol	64, 257
Ethanolamine-o-phosphoric acid	142
Ethyl acetate	47
Ethyl phosphate	284
Factors (chemically unidentified)	
Antibacterial	234, 263
Anti-Pasteur effect	60
Antistiffness	214
Antithyrotoxic	21
Basophilic	275
Cellulose digestion promotion	124, 195, 215, 228
Chick growth	163, 227
Citrate oxidation	72
Citrovorum	38, 96
Corynebacterium diphtheriae	40, 41, 222
Formic hydrogenlyase cofactor	141
Hamogen	149
Lactobacilli	110
Lactobacillus casei	213, 43
Microbacterium flavum	22
Mycobacterium lepraemurium	80

Table VI (Continued)

Constituent	References
Factors (continued)	
Paramecium growth	112
Phytase inhibitor	100
Pyruvate oxidation	89
Schwarz factor 3	226
Staphylococcus toxin promotion	29
Tropical anemia preventative	149
Reversal D-tryptophan inhibition	99
Virus inactivator	249
W	81, 149, 205
Z	239
Flavoprotein	213
Folic acid (vitamin M)	32, 82, 107, 187, 271, 213
Folic acid hexaglutamate	6
Formate	140
Fructose-1,6-diphosphate	136
Fructose-6-phosphate	136
Gamma-aminobutyric acid	142, 148, 203
Globulin (protein)	45
Glucan (1:3 beta linkage)	18, 105, 146, 177, 210, 256, 258
Glucan (1:2 beta linkage)	18, 105, 210

Table VI (Continued)

Constituent	References
Glucosamine	36, 105, 146, 158, 171, 210
Glucose	146, 158
Glucose-1-phosphate	231, 244
Glucose-6-phosphate	231
Glutamic acid	2, 3, 74, 77, 90, 140,
Glutamic acid peptide	107, 142, 182
Glutamine	142, 148
Glutathione	11, 36, 45, 136, 142
Glycerol	30, 268
Glycine	2, 3, 77, 107, 142
Glycogen	171, 176, 184, 191, 231, 256, 258
Guanine	63, 86
Guanine-uridylic acid	63
Guanylic acid	86
Hexosediphosphate	157, 171
Histidine	2, 3, 77, 140, 225, 24, 74, 107, 142
Humine (decomposition product)	8
Hydroxyglutamic acid	182
Inositol (L and meso)	12, 220, 239, 270, 281
Isoamyl amine	108

Table VI (Continued)

Constituent	References
Isocitric acid	
Isoleucine	2, 3, 24, 107, 142 25, 26
Isoleucyl-isovalyl-isoleucyl-leucine (a cyclic tetrapeptide)	217, 218
Ketoheptose monophosphate	171
Lactic acid	268
Lauric acid	285
Lecithin	30, 36, 81, 246
Leucine	2, 3, 24, 77, 107 26, 142, 266
Linoleic acid	30, 285
Lipid	177
Lipoprotein	178
Lumichrome	119, 270
Lysine	2, 3, 26, 77, 241 24, 74, 107, 142
L-Lyxose	86
Mannan	144, 171, 177, 191, 209, 256, 258, 146, 147
Mannose	146
Methionine	3, 24, 26, 42, 107, 142
Meta phosphate	276
Mycetin	144, 146, 147

Table VI (Continued)

Constituent	References
Nicotinic acid (niacin)	12, 107, 254, 269, 270 65, 281
Nicotinic acid amide (nicotinamide)	149
Nitrogen	91, 105, 287, 288
Norleucine	8
Nucleic acids	51, 86, 146, 257
Nucleoproteins	83
Yeast nucleotides (guanylic acid, adenylic acid, cytidylic acid, uridylic acid)	86
Oleic acid	30
Ornithine	142
Oxyglutamine	8
Oxyproline	8
Oxaloacetic acid	71
Palmitic acid	30
Pantothenic acid	12, 220, 270, 274, 281
Para-aminobenzoic acid	1, 48, 81, 134, 202, 281
Para-aminobenzoic acid peptide	202
Para-hydroxybenzoic acid	48
Para nucleoprotein	45
Pentosan	171
Phenylacetylcarbinol	231

Table VI (Continued)

Constituent	References
Phenylalanine	2, 76, 77, 107 24, 25, 26, 142
6-Phosphogluconate	216
Phosphoglyceric acid	136
Pigment (pink)	37
Pigment (red)	61
Polyphosphates	58
Proline	2, 3, 77, 142
Provitamins D_2 and D_3	149
Pteroylheptaglutamic acid	75, 202
Pyridoxal	12, 197, 199, 281
Pyridoxal phosphate	198
Pyridoxamine	12, 197, 199
Pyridoxamine phosphate	198
Pyridoxine	12, 107, 197, 270, 281
Pyrimidine	12
Pyruvate (pyruvic acid)	4, 71, 268
Ribitol (adonitol)	171
Riboflavin	65, 107, 269, 270, 281
Riboflavin-phosphoric acid	213
Ribonucleic acid	36, 136
Ribonucleoproteins	122

Table VI (Continued)

Constituent	References
D-Ribose	70, 86, 171
Saccharose	253
Serine	2, 3, 77, 135, 142
Squalene	30
Starch	158
Stearic acid	30
Streptogenin	214
Succinnic acid	44, 253
Taurine	142
Threonine	2, 3, 24, 25, 26, 107, 142
Thiamine (aneurin)	107, 156, 254, 269, 270 65, 113, 281
Thiamine pyrophosphate	138
Thiazole (substituted)	12
Thiochrom	213
Thiomethyl pentose	
Trehalose	59, 168, 171, 237, 256, 257 126, 184, 236
Trimethylamine	108
Tryptophan	2, 3, 24, 25, 26, 107
Tyrosine	2, 3, 74, 76, 77, 140 24, 26, 142
Tyrosine codecarboxylase	264

Table VI (Continued)

Constituent	References
Uracil	70, 86
Uridine triphosphate	180
Uridylic acid	63, 86
Valine	2, 3, 24, 77, 107 25, 26, 142
Vitamin B_3	81, 149, 205, 254
Vitamin B_4	81, 149, 254
Vitamin B_5	81, 149, 205, 254
Vitamin B_7	149, 205
Vitamin B_{10}	214
Vitamin B_{11}	214
Vitamin B_{12}	78, 281
Vitamin B_c conjugate	188, 205
Vitamin B_x (anti-gray hair factor)	149, 205
Vitamin E (tocopherol)	149
Vitamins L_1 and L_2	149, 205
Vitamin M (folic acid conjugate)	149, 205
Vitamin T	242
D-Xylose	158
Yellow protein	14
Zymocasein protein	268

4. It is not adsorbed on Nalcite HCR or IRA-410 ion exchange resins.
5. It is destroyed by ashing.
6. It is not precipitated by protein precipitants.
7. It does not appear to be an amino acid.
8. It does not appear to be a B-complex vitamin.

One of the bottlenecks in the study of cellulolytic bacteria has been the lack of chemically defined propagation media. Many problems in ruminant nutrition and ruminant chemistry depend upon the nutrition of the bacteria whose enzymes degrade the cellulosic roughages in the diet of the rumen animal.

A part of this investigation was devoted to the development of a transparent isolation medium for cellulolytic rumen organisms using carboxymethylcellulose as the carbohydrate source (261). Water-clear carboxymethylcellulose (94) media were also used to test some of the characteristics of the unknown cellulolytic stimulation factor in food and fodder yeast. It was hoped that these media would facilitate the study of the requirements of rumen organisms.

By knowing the metabolic requirements of the cellulolytic bacteria and the pathways through which cellulose digestion occurs, it should be possible greatly to facilitate the improvement of ruminant nutrition with the goal of providing more abundantly, and at lower cost, some of the animal proteins which are so vital to the health and well-being of the people of the world.

III. MATERIALS

A. Special Materials

1. Eugon agar

The Eugon agar medium used in isolating cellulolytic rumen organisms was obtained from the Baltimore Biological Laboratories, Inc., Baltimore 18, Maryland, and had the following composition:

Tripticase	15 g.
Phytone	5.0 g.
Sodium chloride	4.0 g.
Sodium citrate	1.0 g.
Sodium sulfite	0.2 g.
L-Cystine	0.2 g.
Dextrose	5.0 g.
Agar	15.0 g.

2. Cellulose

CMC-70 L was used as a source of cellulose for the cellulolytic bacteria (261). It is one of the sodium salts of a carboxymethylcellulose (94). The CMC-70 L has a low viscosity in solution and the number of sodium carboxymethyl groups introduced into the cellulose molecule is in the range of 0.65 to 0.85 substitutions per glucose residue. This cellulose gum, sold under the trade name CMC, was obtained from the Hercules Powder Company, Cellulose Products Department, Wilmington 99, Delaware.

Alphacel is a non-nutritive cellulose powder obtained from the Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

Solka floc is a finely ground cellulose flour. It was used as a source of cellulose for the cellulolytic bacteria. It was obtained from the Brown Company, Berlin, New Hampshire.

3. Rumen liquor

This material was used as a source for isolating cellulolytic bacteria and was used as a source of growth factors for the propagation medium. Rumen contents obtained from a fistulated Holstein cow were filtered through four thicknesses of No. 50 grade cheesecloth and only the liquid portion was used. This was preserved in thermos bottles when used for inoculum. The cow was kept and maintained on a regular dairy ration at the Iowa State College Dairy Farm.

4. Alcohol

Unless specified otherwise the alcohol used in this investigation was 95 per cent commercial ethanol.

5. Ion exchange resins

The anion exchange resin used was Amberlite IRA 410. The cation exchange resin used was Nalcite HCR. These were obtained from the General Filter Company, Ames, Iowa.

6. Phytone

This is a papaic digest of soya meal. It was used as a nitrogen source and tested for growth factors for cellulolytic digestion. It was obtained from the Baltimore Biological Laboratories, Inc., Baltimore 18, Maryland.

7. Trypticase

Trypticase was used as a source of organic nitrogen and to test for growth factors for cellulolytic digestion. It is a peptone derived from casein by pancreatic digestion. It was obtained from the Baltimore Biological Laboratories, Inc., Baltimore 18, Maryland.

8. Norite A

This is a decolorizing carbon used for adsorbing growth factors. It was obtained from the Pfanstiehl Chemical Company, Waukegan, Illinois.

9. Vegetable oil

This was used in attempting to extract the flavors from yeast. It was a corn oil with the trade name Wesson oil and was obtained through regular retail channels.

10. Yeast

Several sources of yeast were used in this investigation. Yeasts were used in an attempt to isolate a dry, bland protein concentrate and also used as a source of growth factors for the stimulation of cellulose digestion by rumen organisms.

The bakers' pressed yeast that was used contained approximately 69.6 per cent moisture and 9.3 per cent nitrogen on a moisture free basis. It was obtained in pound cakes from the Purity Bakery, Ames, Iowa.

Dry torula fodder yeast was used as a source of growth factors and in several attempts to prepare bland proteins. The analysis was: protein, minimum, 45 per cent; fat, minimum, 0.5 per cent; fiber, maximum, 3.0 per cent; moisture 8 per cent; and nitrogen free extract, minimum, 35 per cent. This was obtained from the Lake States Yeast Corporation, Rhinelander, Wisconsin.

Frozen torula yeast was used as a protein source. It contained 75.8 per cent moisture and 10.1 per cent nitrogen on a moisture free basis.

The commercial yeast extract used during this investigation was powdered Difco Bacto Yeast Extract and was obtained from the Difco Laboratories, Detroit 1, Michigan.

B. Chemicals

All chemicals used during this investigation were of C. P. grade. They were obtained from regular commercial sources.

IV. METHODS

A. Production of Torulopsis utilis

A slant culture of T. utilis obtained from Dr. L. A. Underkofler of the Iowa State College Chemistry Department was used as inoculum. This culture was carried on dextrose agar slants of the following composition: dextrose, 40 g.; agar, 15 g.; yeast extract, 5 g.; water, 1 l. The inoculum was built up in steps by transferring from the slant into 50 ml. Erlenmeyer flasks containing 20 ml. of sterile glucose-salts medium (183). After incubation, each 20 ml. of culture was used to inoculate a 500 ml. flask containing 200 ml. of glucose-salts medium. These in turn were transferred to 4 l. Fernbach flasks containing 1 l. of medium. All flask cultures were incubated at 30° C. on a shaker.

When larger quantities of cells were required, 4 l. of torula culture were used to inoculate 25 gal. of sterile medium in a 75 gal. capacity fermentor. It was equipped with a steam jacket, agitator and air sparger. Cotton seed oil was used as an antifoam agent.

The glucose-salts medium contained the following ingredients:

Glucose	70 g.
Sodium chloride	2 g.
Magnesium sulfate	2 g.
Calcium chloride	0.4 g.
Potassium pyrophosphate	2 g.

Ferric pyrophosphate	0.1 g.
Ammonium chloride	0.15 g.
Copper sulfate	trace
Tap water	1 l.

A sucrose-salts medium was used for the large scale fermentations of T. utilis. Its composition was as follows:

Sodium nitrate	2.0 g.
Potassium chloride	0.5 g.
Magnesium sulfate.$7H_2O$	0.5 g.
Ferrous sulfate.$7H_2O$	0.01 g.
Potassium phosphate (monobasic)	.01 g.
Potassium phosphate (dibasic)	1.0 g.
Sucrose	50.0 g.
Corn steep liquor	2.0 ml.
Tap water	1.0 l.

Harvesting the cells from the flask cultures for use in exploding experiments was simply a matter of centrifuging at about 2000 r.p.m. in a bucket type International centrifuge. The cells were then washed two or three times with ice cold water to remove the medium. The cells were then resuspended in cold water or 0.95 per cent saline to the desired concentration for exploding. An electrically driven Sharples super centrifuge was later found to be more practical than an International basket centrifuge or a plate and frame filter press for harvesting the cells from the 25 gal. batches.

B. Taste Panel

The taste testing of the various protein concentrates was done by comparing the blandness and taste of the dried products with a sample of dried, pressed bakers' yeast or dried torula yeast. The panel varied from a minimum of two persons for the bad tasting products to as many as 25 persons for the blandest proteins.

C. Anaerobic Procedures for Cellulolytic Rumen Bacteria

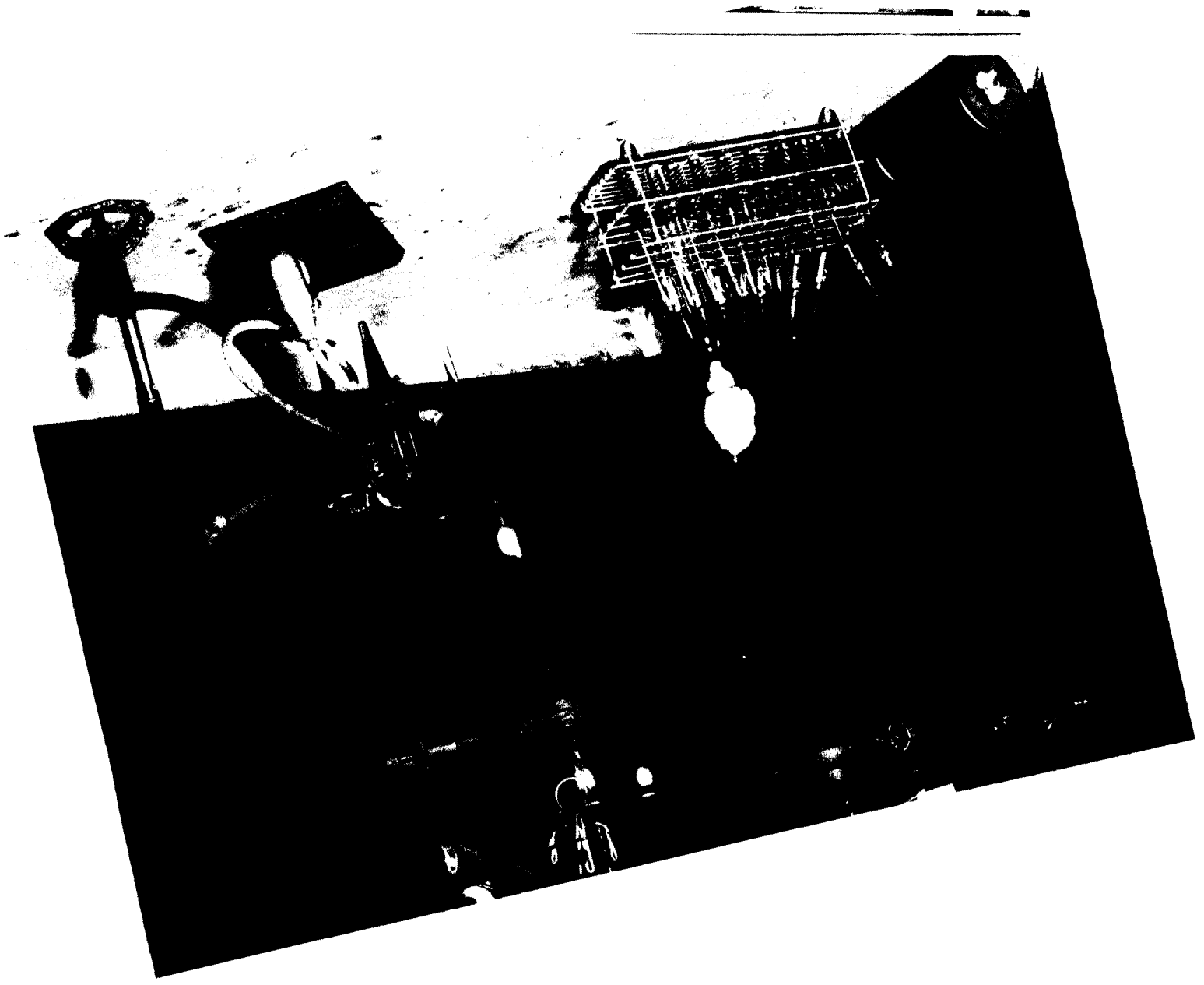
1. Sterilization of medium

The medium was dispensed into 15 x 125 mm. test tubes. Each tube was fitted with number 0, one hole, rubber stopper with a glass rod inserted. About 3 or 4 cm. of the glass rod protruded from the bottom of the stopper. As shown in Figure 1, this allowed the stoppers to be set up on the top edge of the tubes without falling off during sterilization. The test tubes, in wire test tube racks, were sterilized in the autoclave for 15 minutes at 15 p.s.i. steam pressure. After shutting off the autoclave and allowing the pressure to be lowered gradually, but while still hot, the autoclave was opened and the stoppers replaced tightly in the test tubes. This enabled both sterile and anaerobic conditions to be maintained.

2. Dilution of inoculum

It was necessary to dilute the rumen liquor and bacterial cultures before using them as inoculum in isolation experiments. The dilutions were performed in 100 ml. square milk dilution bottles. This was

Figure 1. Sterile transfer chamber and equipment used for anaerobic inoculation of rumen organisms



initiated by adding 1 ml. of fresh rumen liquor or active cell cultures to 99 ml. of 1/15 M phosphate buffer of pH 7. Appropriate aliquots were then transferred from each dilution bottle in sequence until the desired dilution was reached, i.e., 10^{-2}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-9}. Anaerobic conditions were maintained by gassing each solution thoroughly with carbon dioxide. Resazurin dye was used in the buffer to indicate anaerobic conditions.

The device for delivering sterile carbon dioxide and transferring the dilution medium is shown in Figure 1. It consisted of a glass tube 1.5 x 25 cm. packed with 10-24 mesh activated carbon. It had a glass wool plug at each end. One end of the tube was fitted with a one-hole rubber stopper into which fitted a 2 ml. short pipet. The other end was fitted with a glass T to fit the carbon dioxide inlet and a rubber bulb. The dilution buffer was first gassed until the resazurin showed reducing conditions. The culture was then pipetted into the first dilution bottle. The bottle was again gassed. The gas supply was shut off and the rubber bulb manipulated in such a manner as to draw 1 ml. of material into the pipette for transfer to the next bottle. The pipette was rinsed with each dilution. Sterilization of this apparatus was done in the autoclave. Aseptic conditions were maintained by performing all operations in the sterile transfer box shown in Figure 1. A 25-watt germicidal lamp was used to sterilize the chamber.

3. Inoculation procedure

It was found most desirable to grow the cultures used for inoculating the various test media in 15 ml. conical bottomed centrifuge

tubes. The media were kept anaerobic by using rubber centrifuge caps during incubation. This made it possible to centrifuge the cells and wash them twice with buffer without having to remove them from the tube they were grown in. Finally the washed, centrifuged cells were re-suspended in buffer, gassed with carbon dioxide to maintain anaerobic conditions, and used as inoculum.

The inoculum was transferred by inserting a sterile glass tube and stopper arrangement, similar to that of a wash bottle, in the centrifuge tube containing the inoculum. This is shown in Figure 1. The inoculum delivery tube was so constructed that 20 drops were equivalent to 1 ml. By attaching a rubber bulb to the inlet of the inoculum tube and tightening a clamp gradually on the rubber bulb, it was possible to maintain anaerobic conditions and deliver the inoculum drop-wise with perfect control.

4. Determination of results

In the various tests for the growth factor which stimulated cellulose digestion, growth of bacteria was taken as the criterion for the presence of the factor and non-growth of bacteria indicated the absence of the growth factor. The amount of growth was compared with the control media by rating it from 1+ to 4+. It was possible to do this visually with the transparent CMC media used in this investigation.

It would have been possible to check growth by turbidimetric assay using a photoelectric colorimeter, except for the fact that the growing bacteria also discolored the media. A completely colorless medium was

not developed in this investigation. However, it is felt that this would be possible if purified factors were used instead of mixed natural materials, i.e., yeast extracts.

D. Analytical Methods

1. Kjeldahl analysis

A micro-Kjeldahl technique was used. The sample size, mercuric sulfate catalyst and digestion procedure of Hiller, Plazin and Van Slyke (98) were used. The ammonia was distilled, absorbed in 4 per cent boric acid and titrated with 0.02 N hydrochloric acid as recommended by the Association of Official Agricultural Chemists (10) and Yuen and Pollard (286).

2. Biuret test

The biuret test was used as a qualitative test for protein in the yeast exploding experiments. The procedure used was adopted from Fine (68), Hiller (97), Robinson (206, 207), Snow (232) and Stiff (238).

3. Determination of total explodate nitrogen

The amount of nitrogen released or exploded from the yeast cells as water soluble nitrogen compounds during the various processes was determined by centrifuging a 25 ml. sample of the homogeneous exploded yeast slurry and decanting the centrifugate into a 50 ml. volumetric flask. The residue was then resuspended in about 20 ml. of 0.95 per cent sodium chloride solution and recentrifuged. The centrifugates

were combined and made up to 50 ml. The Kjeldahl-nitrogen was analyzed using 1 ml. replicates. This water soluble nitrogen was used to calculate the per cent of the total nitrogen which was exploded out of the cells and found in the cell free centrifugate (explodate).

4. Determination of protein nitrogen in explodate

A 15 ml. sample of the cell free explodate (described in part 3 above) was placed in a centrifuge tube and 30 ml. of 95 per cent ethanol added. This precipitated the protein. Trichloracetic acid was not used to precipitate the protein because the heated yeast proteins used in this investigation usually formed a colloidal suspension instead of a precipitate with 10 per cent trichloracetic acid. After thorough mixing with alcohol, the protein sample was allowed to stand for 20 minutes to allow the protein to coagulate. The tube was then centrifuged, the alcohol decanted off, and the protein precipitate thoroughly drained. The protein was then resuspended in distilled water, made up to 5 ml. volume and the Kjeldahl-nitrogen determined. This precipitated protein was calculated as per cent nitrogen on the basis of the total nitrogen in the original yeast slurry.

V. EXPERIMENTAL

A. Yeast Exploding Procedures

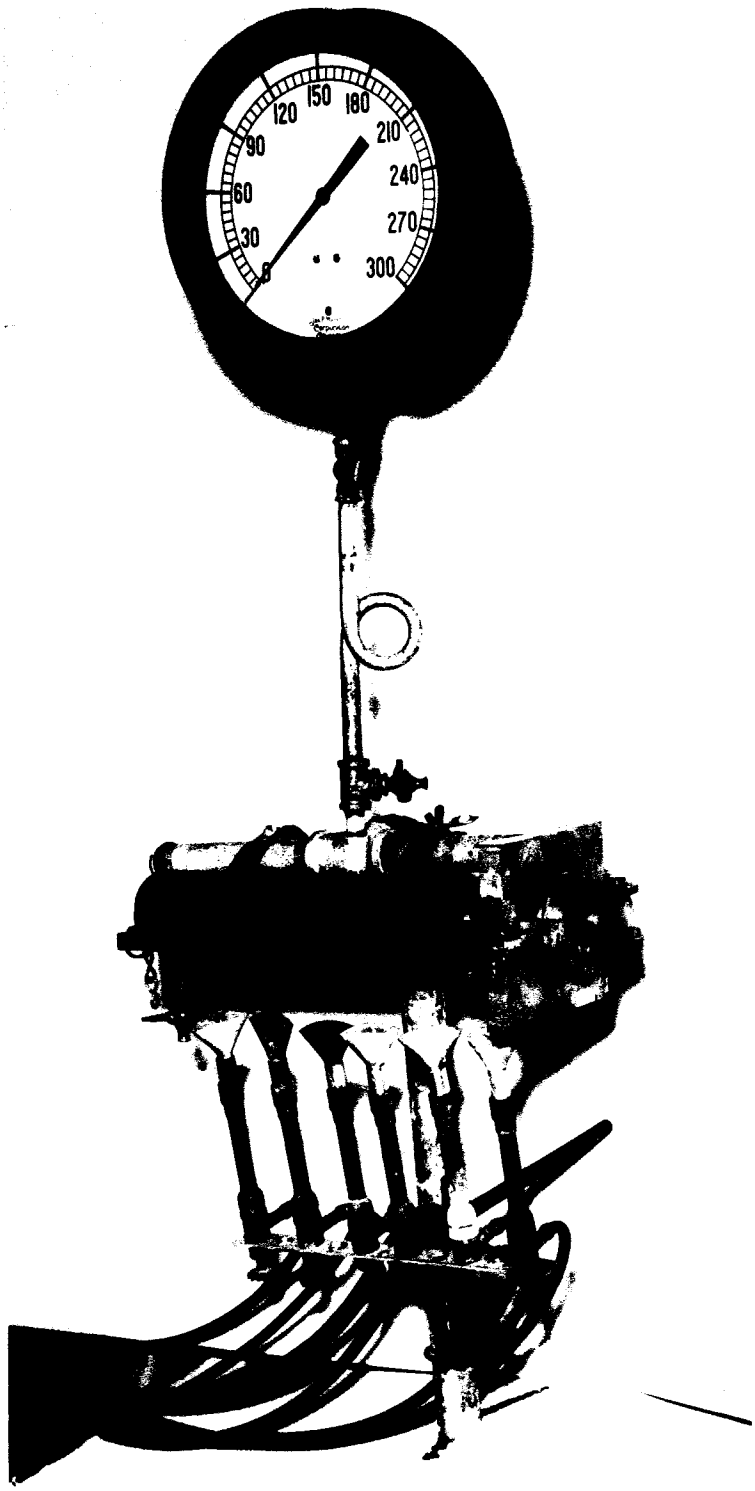
In order to remove the taste-producing small molecules from yeast protein, it is, first, necessary to make the yeast cell wall more permeable or rupture it in order to make the cell constituents accessible for processing. A part of this investigation was concerned with the possibility of cell rupture by a method similar to that used in making "puffed wheat" (7, 160, 196).

The principle involved is to heat a slurry of yeast in a closed system to a temperature above the boiling point of water, or cell liquids, at atmospheric pressure. The system is then opened in such a manner as to allow the external pressure of the cells to escape faster than the internal pressure can dissipate through the cell wall. The resulting pressure differential, when it is greater than the elastic limit, should break the cell wall. One of the purposes of this investigation was to see whether this principle would be effective in a time, temperature and pressure range which would make the process feasible in the commercial preparation of protein concentrates from yeast. Throughout this investigation the term "exploding" was used to signify the process of heating a yeast cream in a closed system, followed by a quick release of the pressure.

1. Quick opening autoclave

The quick opening autoclave shown in Figure 2 was made in order to test whether yeast cell rupture would occur in a batch process similar

Figure 2. Quick-opening autoclave for exploding yeast


30
60
90
120
150
180
210
240
270
300

to that used in making puffed wheat (7). The autoclave was made of 2-in. iron pipe and mounted so that it could be tilted easily for filling. The procedure was initiated by pouring a water slurry of yeast into the autoclave. The quick opening door was then closed and forced against the O ring gasket by tightening the two bolts. The autoclave was then tilted back to a horizontal position. The battery of Bunsen burners and a wooden explosion barrier, faced with transite for fire resistance, were then placed into position. The receiving vessel for the exploded material was then positioned. A 5-gal. bucket was used to catch the material when the quick opening trap door was used to explode all of the material instantaneously. Beakers were used when the material was allowed to escape through the drain cock orifice.

When everything was in its proper place, the burners were lighted. It was possible to control the burners individually by means of a valve manifold. When the pressure gauge indicated that a small amount of pressure was developing, the bleeder valve was opened and the air allowed to bleed from the system. The valve was then closed and the heating continued. After the system had reached the desired steam pressure, the lever on the side of the autoclave was tripped allowing the door to fly open and release the contents into the bucket. At pressures above 60 p.s.i., the O ring gasket was invariably disintegrated by the explosion.

For experiments where the yeast slurry was allowed to escape through the drain cock orifice, a piece of pipe, slotted at the end,

was used for a wrench to open the drain cock. The burner manifold was manipulated in such a manner as to maintain constant steam pressure during the entire time that the yeast slurry was being released.

The first goal was to remove all of the protein from the yeast cell. It was hoped that subsequent treatment would remove the taste-producing small molecules and leave a bland protein concentrate suitable for human consumption. It was, first, desired to find the yeast exploding capabilities and limitations of the apparatus. This was done by determining the amount of Kjeldahl-nitrogen exploded from the cells. In the preliminary trials, microscopic examination was made of the cells using methylene blue as a vital stain. This was done to see what effect exploding had on the viability and morphology of the yeast cell.

Dry torula yeast was used in two preliminary runs with the quick opening autoclave. This was done to check the efficiency of the quick opening valve and see if there was any morphological effect on the dried yeast cells. A 10 g. sample of dry torula fodder yeast (see Materials) was suspended in 30 ml. of water. This was exploded at 5 p.s.i. steam pressure by releasing the trap door as previously described. A 4-minute heating period was required, including time for bleeding air out of the system.

A microscopic comparison between the exploded and unexploded dry torula yeast was made using methylene blue stain. The exploded cells were larger than the unexploded ones. In contrast to the untreated

yeast, a small fraction of the exploded cells did not stain with methylene blue. It was thought that this represented complete emptying of the cell wall contents.

A second run was made. Again, 10 g. of dry torula yeast was used. The exploding temperature this time was the equivalent to 10 p.s.i. steam pressure. In order to insure complete tempering of the cells, a 5-minute holding time at 10 p.s.i. was employed. The microscopic results were the same as in the first test.

Freshly harvested and washed T. utilis cells were made into a 10 per cent water slurry. This slurry was divided. One half was exploded at 30 p.s.i. and one half at 80 p.s.i. steam pressure by releasing the quick opening valve and catching the exploded material in a 5-gal. bucket.

Vital stains of both were similar and indicated that all of the cells were killed. However, only a small number of the cells indicated a change in morphology, i.e., larger size.

Freshly harvested T. utilis cells (75 g. wet weight) from 3 l. of beer were washed with distilled water and resuspended in 125 ml. of distilled water. These cells were exploded at 123 p.s.i. by releasing the quick opening valve. Vital staining with methylene blue indicated that about 25 per cent of the cells were drastically affected. The centers of some of the cells did not take any stain. The nucleus appeared to remain inside these cells but it was free to migrate inside the cell wall. Some of the cell walls seemed to be empty as they

easily became distorted and elongated when moving with the currents in a wet-mount, stained microscope slide. There was a great deal of contrast between wet mounts of exploded cells and similarly mounted live cells and dead cells.

Heat fixed, methylene-blue, stained slides of exploded and unexploded cells presented a great contrast. The unexploded cells retained their cell shape and stained blue. The unexploded cells had the appearance of irregular and shriveled cell debris. No complete cells appeared in the exploded yeast slide. This indicated that something drastic had occurred to the cells during exploding.

A 30-g. sample of freshly harvested, thrice washed, T. utilis cells, suspended in 100 ml. of distilled water, was exploded at 160 p.s.i. by releasing the quick opening valve and catching the material in a 5-gal. bucket. The product was recovered by rinsing it into a beaker. It was readily apparent that there was too much material lost by splattering to get significant quantitative data; however, a check was made to see if any nitrogen compounds were being exploded out of the cells.

Samples of the original unexploded yeast slurry and the exploded material were centrifuged at 2000 r.p.m. for 15 minutes, and the Kjeldahl-nitrogen determined on the centrifugates. The centrifugate from the exploded yeast (hereinafter called explodate) was centrifuged at 12,000 r.p.m. for 20 minutes and a Kjeldahl analysis run on the

twice centrifuged explodate. The nitrogen analysis for the centrifugates are as follows:

Original	0.0 mg. Nitrogen/ml.
Explodate (2,000 r.p.m.)	2.03 mg. Nitrogen/ml.
Explodate (12,000 r.p.m.)	1.41 mg. Nitrogen/ml.

The data indicated that a major change had occurred in the cells. The material precipitated by centrifuging at 12,000 r.p.m. but not at 2,000 rp.m. must be cell fragments or coagulated protein.

Since there was too much material lost during the explosion to get yield data on the exploded cells, a better method of exploding was needed. It was reasoned that exploding the cells from an orifice would afford the same quick release of external pressure as releasing the quick opening trap door. This would make it possible to have closer control of the explosion and make it easier to collect the product. Consequently, a drain cock orifice was installed in the quick-opening autoclave shown in Figure 2.

Freshly-harvested, twice-washed T. utilis cells (150 ml. of slurry) were placed in the quick-opening autoclave and heated to 160 p.s.i. as described earlier. The drain-cock orifice was then cracked allowing the pressure to spray the yeast slurry into a 2 l. beaker. The pressure remained constant until all of the liquid was out; then it rapidly dropped. From the time heating was started until the material was all expelled, 12 minutes had elapsed.

A small amount of loss occurred due to splattering. The material had a burned odor indicating some loss by scorching in the autoclave. The exploded material was recovered by rinsing out the collection beaker, but not the autoclave, and diluted to 150 ml. with distilled water. This was thoroughly mixed and triplicate Kjeldahl samples taken.

The exploded material was then centrifuged at about 2,000 r.p.m. for 20 minutes. The solids separated nicely leaving a dark brown No. 1 centrifugate of 120 ml. volume. The solids were resuspended in 100 ml. of distilled water and again centrifuged to obtain No. 2 centrifugate. This procedure was repeated to obtain No. 3 centrifugate. Finally, centrifugates 2 and 3 and the resuspended solids were each made up to 100 ml. with distilled water. Kjeldahl determinations of each were made in order to follow the nitrogen distribution. This is resolved in Table VII to show the water-soluble, cell-free nitrogen in the centrifugates and the residual nitrogen left in the cell debris.

Microscopic analysis of the exploded material, using wet mount and fixed slides both unstained and stained with methylene blue, revealed that a considerable change had occurred in the cells. The homogeneous exploded material when examined by stained, wet-mount procedure revealed quite a number of cell-shaped particles. A small amount of these cell-shaped particles stained uniformly like dead cells. However, most of them did not take stain in the center. Unstained cell centers seemed to have nuclei floating around in them. These unstained cells deformed and elongated in the currents of the liquid on the slide. A large number of uniformly stained, irregular particles were present.

Table VII

Nitrogen distribution in exploded yeast wash water

Explodate fraction	Nitrogen in fraction in mg.	Per cent of total explodate nitrogen
No. 1 centrifugate	447	51.7
No. 2 centrifugate	96.5	11.1
No. 3 centrifugate	48.5	5.6
Semi-total	592.0	68.4
Residual cell Nitrogen	281	32.5
Grand total	873.0	100.9
Homogeneous exploded material	865.0	100.0

In contrast to this the unexploded starting material revealed a typical yeast morphology with nearly all of the cells alive and no contamination or extraneous particles present. The cells were more rigid and didn't distort in the liquid currents of the wet-mount slide.

The three centrifugates and the residual cell material exhibited no whole cell structures. The centrifugates had only a few very small particles in them and the precipitated cell matter was observed to be irregularly shaped debris. Apparently the force of centrifugation had collapsed the cell walls which had appeared in the homogeneous exploded material.

In order to gain some insight regarding the relative importance of the heating effect on nitrogen release from yeast cells in these exploding experiments, two runs were made with bakers' yeast at 160 p.s.i. The cooking time of one run was 5 minutes longer than the other. All other conditions were held constant. A 200-g. sample of pressed bakers' yeast was suspended and made up to 1 l. volume with distilled water. Run No. 1 was made with 300 ml. of the yeast cream in the quick-opening autoclave. The time from burner lighting until 160 p.s.i. pressure was reached was 11 minutes. The drain cock orifice was cracked, and it required 2 minutes for the yeast to be expelled. The recovered exploded material was made up to 500 ml. with distilled water. Run No. 2 was identical to No. 1 with the exception that the pressure was maintained at 160 p.s.i. for 5 minutes to increase the cooking time. A 200 ml. volume of exploded material from each run was washed and centrifuged four times and the Kjeldahl

determination of nitrogen made for each fraction. Table VIII shows the nitrogen distribution. The data show that an increase in heating time does increase the amount of nitrogen released from the cell.

In order to find out more about the effect of exploding versus the mere heating of yeast, an experiment was run by heating the yeast cream to 160 p.s.i. in the quick-opening autoclave and then cooling it in the autoclave to room temperature before allowing it to drain from the autoclave. This procedure should result in cooling the cell liquids below the boiling point and eliminating the exploding effect. The change in steam pressure with time is recorded in Table IX.

The cell free explodate contained 46.5 per cent of the total nitrogen. This contrasts with 63.9 per cent for the previous experiment with 11 minutes' heating time plus 2 minutes' releasing time. This would seem to indicate that exploding did have some, but not all, of the effect in releasing nitrogen compounds from the cell. It must be borne in mind, however, that there was a difference in the heating time, and the rapid cooling of the autoclave with the resulting vacuum possibly having had the same exploding effect as release from the orifice.

Another experiment was run to test whether fast-cooling at constant pressure, followed by slow pressure release to eliminate exploding, would give an indication of the difference between the cooking and exploding effects. A slurry of bakers' yeast was placed in the autoclave and heated in the usual manner. The pressure had risen to

Table VIII

Change in yeast explodate nitrogen distribution with heating time[a]

Run no.	Fraction of sample	Volume ml.	Total N mg.	Per cent of total N
1	Centrifugate no. 1	195	244	53.3
	Centrifugate no. 2	190	41.8	9.1
	Centrifugate no. 3	200	7.1	1.5
	Centrifugate no. 4	200	none	none
	Semi-total		292.9	63.9
	Residue	500	166.0	36.1
	Total nitrogen		458.9	100.0
2	Centrifugate no. 1	195	335	67.2
	Centrifugate no. 2	160	36.2	7.3
	Centrifugate no. 3	180	24.5	4.9
	Centrifugate no. 4	150	2.3	0.5
	Semi-total		398.0	79.9
	Residue	500	101.0	20.1
	Total nitrogen		499.0	100.0

[a]Run No. 2 was heated 5 minutes longer at 160 p.s.i. than Run No. 1.

Table IX

Change of pressure with time in autoclave

Pressure in p.s.i.	Time in min.	Pressure in p.s.i.	Time in min.
0	0	160	8.166
5	2	150	8.5
5	3	135[b]	9
20	4	115	10
45	5	80	11
70	6	15	12
105	7	0[c]	12.75
150[a]	8		

[a]Turned off heat.

[b]Cooling water turned on autoclave.

[c]Material collected.

160 p.s.i. at 8.5 min. and a sample was collected by cracking the drain cock orifice. Carbon dioxide pressure of 160 p.s.i. was then turned on by means of a polyethylene tube connecting the air bleeder valve and a carbon dioxide tank. The pressure was kept constant while tap water was used to cool the autoclave. The pressure was then released gradually as indicated in Table X.

The final sample shown in Table X had less alcohol precipitable protein than the first sample. This seems to indicate a rather rapid disruption of protein molecules by the 160 p.s.i. steam in the few minutes between the removal of the first sample and the time the autoclave was cooled. Since the pressure was constant during the cooling period, the additional heat should be the major factor contributing to protein degradation.

An experiment was run to see if exploding yeast with carbon dioxide pressure alone, without the use of heat, had any effect on the yeast cells. A 300 ml. sample of bakers' yeast cream (200 g. per l.) was placed in the quick-opening autoclave. A polyethylene tube was used to connect the air bleeder valve with a carbon dioxide tank. A pressure of 160 p.s.i. was introduced into the autoclave and maintained while the yeast was being released through the drain cock orifice. The sample collected was thoroughly mixed and an aliquot centrifuged at approximately 6,000 r.p.m. No nitrogen was found in the centrifugate by biuret test or Kjeldahl nitrogen determination.

Table X

Rapid cooling with constant autoclave pressure

Pressure in p.s.i.	Time in min.	Comment	Per cent nitrogen in explodate	Per cent nitrogen alcohol precipitated [a]
0	0		0	0
160	8.5	Sample taken	43.5	33.5
160	10.5	Cooling water started		
160	15	Autoclave at room temperature	pressure release initiated	
60	100			
5	120			
0	130	Final sample taken	57.5	12.1

[a]The fraction of nitrogen in the explodate which is alcohol precipitable (protein nitrogen) is given in the last column. It is reported as per cent of the total nitrogen in the original homogeneous sample.

Microscopic examination of vitally stained cells indicated that the viability was unaffected by the carbon dioxide's "exploding." This indicates that external pressure alone, at least in the 160 p.s.i. pressure range, has little influence on the unheated yeast cell.

A run was made in the quick-opening autoclave to secure additional data for each of a series of pressures. This was done to duplicate previous experiments and to get additional data for differentiating the effects causing nitrogen release from the cells.

A slurry of bakers' yeast was placed in the quick-opening autoclave and heating started as previously described. Samples were taken at regular pressure intervals as shown in Table XI. The amount of cell free nitrogen in the explodate is shown in the third column of Table XI. It is given as per cent of the total nitrogen in the homogeneous exploded sample. Steam pressure in p.s.i. is plotted against the per cent nitrogen released from the cells in Figure 3. The amount of nitrogen released varies directly with the steam pressure.

It was realized that there were two independent variables in these batch exploding experiments--heating time and temperature. This made it almost impossible to delegate correctly the release of cell nitrogen to the heating or exploding effects.

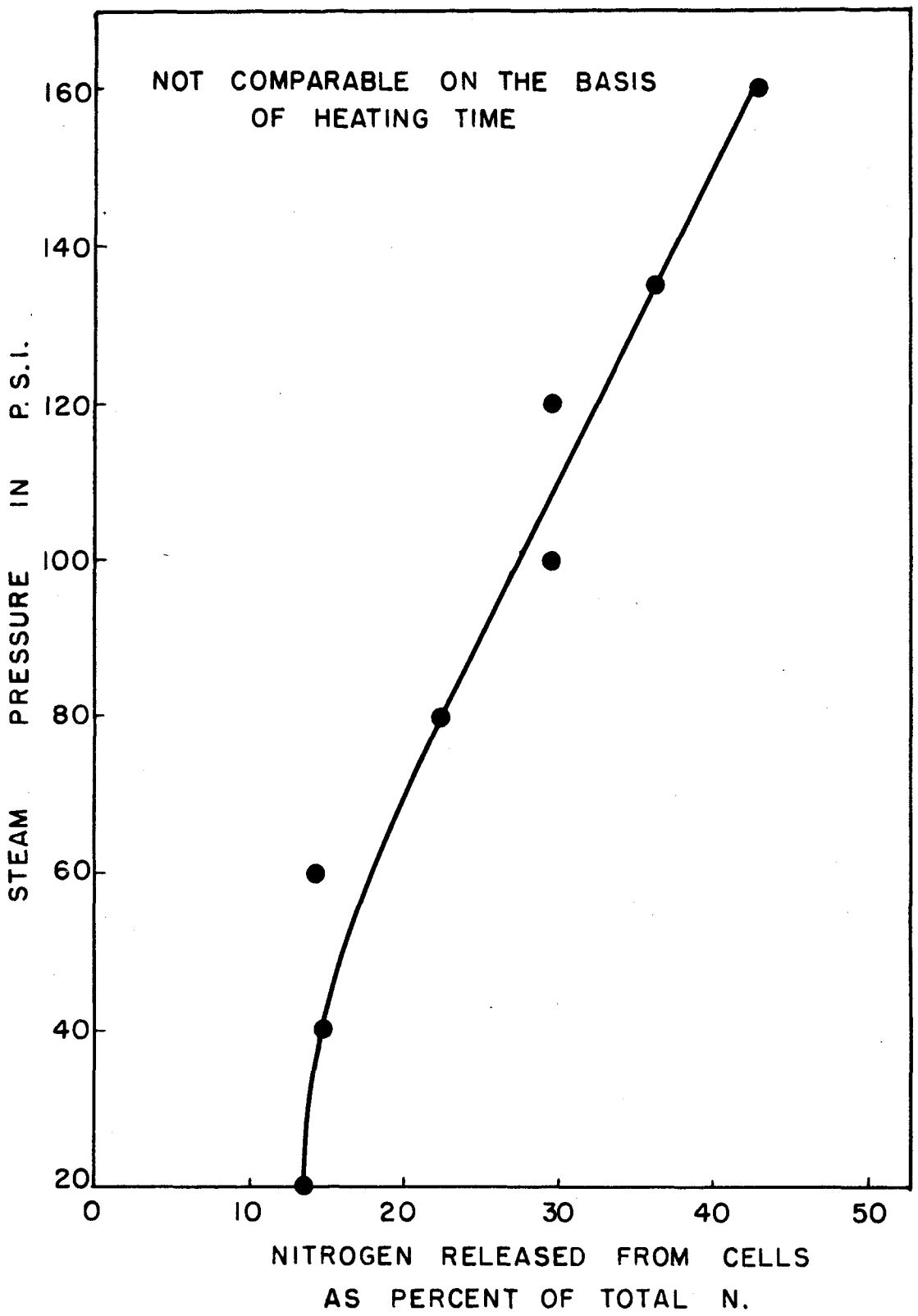
Before it can be said with certainty that the exploding effect and not the cooking heat is the major factor in the release of nitrogen from the cells, it will be necessary to have exact control over the cooking time and this cooking time must be reduced to a minimum. This

Table XI

Nitrogen release from yeast exploded at various pressures

Pressure in p.s.i.	Heating time in min.	Per cent nitrogen in explodate	Odor
0	0	0	yeasty
20	3.63	13.5	yeasty
40	5.66	14.7	yeasty
60	6.41	14.1	yeasty
80	7.58	22.3	yeasty
100	8.75	29.5	yeasty
120	9.75	29.5	yeasty
140	10.50	36.0	yeasty
160	12.50	42.8	scorched

Figure 3. Nitrogen released from yeast cells exploded from a quick-opening autoclave at various steam pressures


NOT COMPARABLE ON THE BASIS
OF HEATING TIME
STEAM PRESSURE IN P.S.I.
160
140
120
100
80
60
40
20
0
10
20
30
40
50
NITROGEN RELEASED FROM CELLS
AS PERCENT OF TOTAL N.

close control is impossible in the batch-type, quick-opening autoclave where the heat is supplied by direct flame. The heat transfer is too slow and there are too many variables for it to be accurately controlled.

The ideal situation would be to develop the internal pressure of the yeast cells instantaneously and eliminate the heating effect entirely. This would allow the internal pressure to expand and break the yeast cell with a minimum of harm to the heat labile substances. Torrington (254, 255), Fraser (73), Milner, et al. (165) and Wallerstein (268) were aware of this problem and met with little success when they tried to rupture yeasts by building up the external pressure on the cells. Torrington (254, 255) had a measure of success by building up the internal carbon dioxide pressure in the cells. The process, however, is expensive and time-consuming when the proper amount of time for pressure equilibration in the cells is considered.

2. Exploding yeast with a continuous, direct-heated exploder

To be commercially feasible, a yeast exploding process should, first, be safe to operate and produce a protein concentrate which will be safe to consume. It must produce a high-quality product which will fill the consumer's needs. It must be able to produce the high volume of product necessary to insure a low unit cost and a high compensation for the time, labor and capital invested. It is desirable that the product should be able to meet competition on all aspects of safety, quality, quantity and unit cost.

Realizing that a continuous exploder would be the most desirable, several models were constructed. By operating a continuous exploder under steady-state conditions it is possible to have an accurate control of time, temperature and material flow and have more of a chance to achieve reproducible conditions.

The first successful continuous device for exploding yeast consisted of a 20-gal. tank made of extra heavy pipe fitted with an agitator and various stop cocks as shown in Figure 4. There was a copper tube (1/4 in.) leading from the yeast reservoir tank to a heat exchanger. There was a valve in this line and a pressure gauge as shown in Figure 4. The heat exchanger was not the same as in Figure 4, but consisted of a 17-in. section of black iron pipe through which the yeast passed. The pipe was heated directly by means of a battery of Bunsen burners. A 6-in. piece of 1/4 in. diameter copper tubing was fastened to the outlet end of the heat exchange pipe by an appropriate union. A brass stop cock was fastened to the copper tubing for an outlet orifice. A thermometer well was made by silver soldering a short length of copper tubing to the outlet tube. The outlet tube was insulated with asbestos.

This heat exchanger was difficult to control because any momentary interruption of the fluid flow caused the yeast slurry to become super heated and form steam pressure greater than the reservoir pressure. As a consequence, all of the yeast slurry in the line backed into the reservoir. Due to this difficulty, only one run was made using the

direct fired heat exchanger. The results obtained, however, are especially valuable, since they represent the shortest heating times that were obtained in this investigation for the respective temperatures. The procedure was as follows.

A slurry was made with 4 lbs. of pressed bakers' yeast and enough ice cold 0.95 per cent salt solution to bring the volume up to 4 gal. This was poured into the reservoir tank and the agitator started. The air line was opened and the pressure in the reservoir built up to air line pressure of 75 p.s.i. The air line was then closed and after making sure the sand bag barricade was in good condition, the nitrogen tank valve was opened and nitrogen pressure used to build up the reservoir pressure to 300 p.s.i. The only purpose of this high pressure was to force the yeast through the heat exchanger at a pressure high enough to keep the slurry in liquid form at the exploding temperature. A good pump would have served the same purpose.

At this point the reservoir drain valve was opened and about a liter of yeast slurry was run through the cold heat exchanger to flush out the system. A sample of this cold yeast slurry, which was forced out the orifice under 300 p.s.i. pressure, was saved and the Kjeldahl-nitrogen determined in the various fractions.

The method of reaching steady state continuous yeast explosion with this direct heated device was as follows. The valve allowing the yeast to flow from the reservoir to the heat exchanger was opened and the stop cock orifice adjusted to allow a rapid flow of yeast into the

receiver. The burners were then lighted and the yeast allowed to flow at a constant rate until a steady state orifice temperature was reached. The heat input remained the same throughout all of the experiment, as the burner adjustments were not changed. Steady state continuous exploding within a temperature range from 100° to 200° C. was achieved by adjusting the yeast flow. This was done by gradually closing the stop cock orifice with a wrench and allowing the heat to reach steady state after each adjustment.

Samples were taken at several different outlet temperatures. The data are tabulated in Table XII. In this table the amount of nitrogen exploded from the cells is shown as per cent of the total nitrogen in the original homogeneous exploded material. With the exception of the 160° C. temperature, there was a regular increase in the nitrogen exploded from the cells with increase in temperature. Since the heating time is very short and relatively similar in every case, the data seem to indicate that an increase of temperature, with corresponding increase in internal cell steam pressure, does cause an increase in nitrogen release.

3. Exploding yeast with a continuous, steam-heated exploder

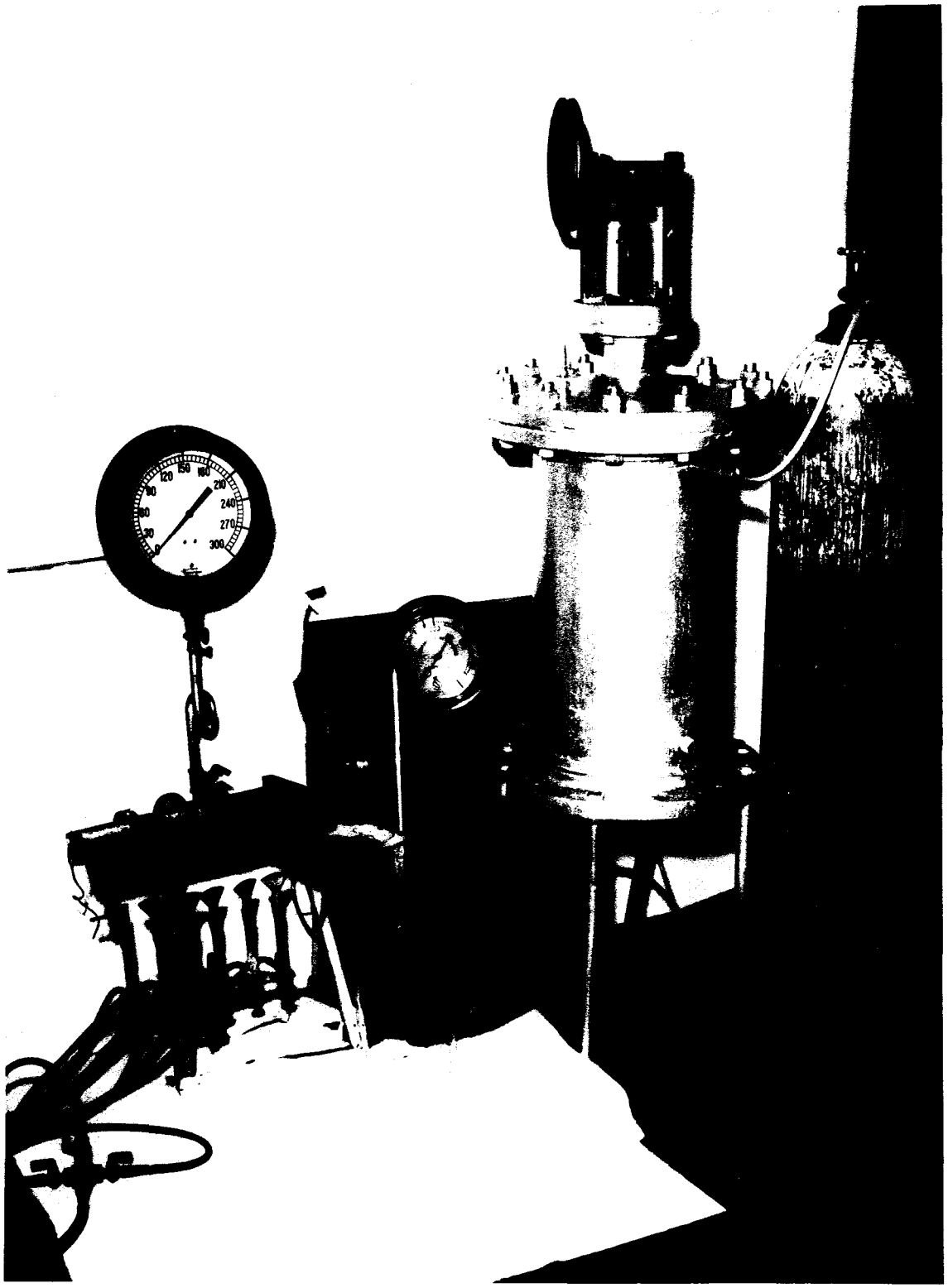
The data from the quick-opening autoclave indicated that pressures higher than the 80 p.s.i. available in the steam line would be required to adequately explode yeast on a continuous basis. The need for more accurate control of the heat to the heat exchanger was pointed out by the difficulty encountered in adjusting the direct-fired heat exchanger to steady-state conditions.

Table XII

Continuous explosion of yeast using direct heat

Sample no.	Heating time in min.	Exploding temperature C.	Per cent nitrogen in explodate	Odor
1	0.163	100	11.5	yeasty
2	0.222	100	9.6	yeasty
3	0.138	120	17.0	yeasty
4	0.052	160	5.5	yeasty
5	0.260	180	19.3	yeasty
6	0.112	200	21.4	burned
7	.....	200	22.6	burned

Figure 4. Apparatus assembly for continuously heating yeast with a steam-heated heat exchanger and releasing or exploding it from an orifice


120
150
180
210
90
240
60
270
30
300
0

The steam-heated, continuous exploder, as shown in Figure 4, consists of a yeast slurry reservoir with a gauge for measuring reservoir pressure, a heat exchanger for heating the yeast, a thermometer for measuring outlet temperatures and a drain cock orifice for releasing or exploding the yeast.

The heat exchanger was constructed from the quick-opening autoclave. The valve door and release were removed and a coil of 1/4 in. copper tubing was inserted. The inlet and outlet to the heat exchanger coil were silver soldered to a blank flange which was bolted on in place of the trap door. In order to generate steam it was, first, necessary to remove the pressure gauge and pour enough water into the autoclave to half fill it. The gauge was then replaced and the Bunsen burners lighted. The air was purged from the system by means of the bleeder valve just below the pressure gauge. Proper adjustment of the flow of gas to the burners by manipulating the valve manifold made it possible to maintain constant steam pressure in the autoclave in the pressure range of 0 to 300 p.s.i., providing the slurry flowing through the coil did not remove the heat faster than the heat flow from the burners through the pipe to the steam chamber.

The exploding procedure was as follows. The valve leading from the reservoir tank to the heat exchanger was closed. The stop cock was then removed from the top of the reservoir. A funnel was placed into this hole and the yeast slurry poured in. The stop cock was replaced and the airline connected to it by means of polyethylene tubing. The agitator was started and the air turned on until the maximum line

pressure was reached in the reservoir. This was usually about 75 p.s.i. The air line was then shut off and the pressure was then increased in the reservoir to a maximum of 300 p.s.i. by the use of bottled nitrogen or oxygen. This pressure was used as the driving force to push the yeast slurry through the heat exchanger. It could have been eliminated by using a gear pump to pump the yeast directly to the heat exchanger. Such a pump was not available.

The orifice and line valves were then opened and after allowing a small amount of yeast to flush out the system, a sample was taken for Kjeldahl-nitrogen determination. The burners under the autoclave were lighted and steam generated to the desired pressure as described above.

The stopcock orifice was used to regulate the flow of yeast to the desired rate. It was necessary that the reservoir pressure be maintained above that in the steam generator to eliminate steam formation in the yeast line. This would cause the yeast to be forced back into the reservoir and stop the flow of yeast through the heat exchanger.

The steam pressure was held constant and the yeast flow also maintained at a constant rate until the orifice thermometer showed that a steady state had been reached. The yeast cream was then allowed to flow at steady-state conditions until two or three times the volume of the heat exchanger had passed through. The volumes of the heat exchanger and outlet orifice were 160 ml. The preliminary material was discarded and a sample at steady state saved for analysis. These data made it possible for the calculation for heating time to be made. Since it was not known how much of the water was being flash evaporated as the

yeast sprayed into the receiver and no flowmeters were available, the Kjeldahl-nitrogen was used to keep track of the rate of flow. The nitrogen content of the original yeast cream and the exploded material were used to calculate the volume flow through the heat exchanger by the following equation:

$$\text{Heating time} = \frac{\text{Volume of heater (collecting time in minutes)(mg. nitrogen/ml. original)}}{\text{(volume of sample)(mg. nitrogen/ml. exploded sample)}}$$

Samples were taken at several different temperatures and the data recorded in Table XIII. The amount of the total nitrogen found in the cell free explodate is given in Table XIII in the column labeled per cent of total nitrogen in explodate. The amount of the cell free nitrogen which was alcohol precipitable is given in the column labeled per cent of total nitrogen ethanol precipitated.

From these data it can readily be seen that there is no correlation between the amount of nitrogen exploded out of the cell and that which is alcohol precipitable. It can also be seen that for the production of a human food, temperatures greater than 160° C. would produce a yeast product with a burned smell unless the heating time was reduced to about 0.25 minutes as shown in Table XII.

It was desired to prove more definitely the true nature of the forces which release nitrogen from the yeast cell during the so-called exploding technique. In order to do this, an experiment was needed where the yeast cream could be heated to the desired temperature and then quickly cooled before it was forced out of the orifice. It was assumed

Table XIII

Continuous explosion of yeast using steam heat

Sample no.	Temperature °C.	Heating time in min.	Per cent of total nitrogen in explodate	Total nitrogen ethanol precipitated	Odor
1	6	0	0	0	unexploded
2	120	0.678	20.4	3.76	yeasty
3	120	0.875	22.7	9.83	yeasty
4	140	1.95	31.5	6.95	yeasty
5	140	5.0	26.8	7.05	yeasty
6	140	10.25	30.0	5.83	yeasty
7	140	15.17	31.4	6.83	yeasty
8	155	0.64	28.9	7.13	yeasty
9	155	4.16	60.5	6.87	burned
10	160	2.90	50.7	6.45	burned
11	170	3.0	burned odor and protein coagulated		
12	180	3.0	burned odor and protein coagulated		

that if yeast exploded, it was from the rapid release of internal cell pressure. The pressure was due to the fact that the internal cell moisture was heated above its vaporizing temperature at the atmospheric pressure in which it was released. The pressure differential was caused by releasing the external pressure faster than the internal pressure could diffuse through the cell wall barrier. If the pressure differential was great enough, that is, higher than a certain critical pressure, the cell would break. By reducing the internal temperature of the heated yeast below the vaporization temperature (100° C.) the exploding phenomenon could be eliminated. This would allow a comparison between the effect of heating alone and heating plus exploding.

In order to cool the hot yeast cream rapidly, a water-jacketed condenser was made to fit the end of the heat exchanger. It was made from 1/4-in. copper tubing. The length, including the part extending from the condenser jacket to the draincock orifice, was 20 inches. A thermometer well was soldered to the outlet end. The volume of the condenser was 10 ml. The draincock orifice was removed from the steam-heated heat exchanger and a union used to fasten the condenser in its place. The stopcock orifice was tightened into place at the outlet end of the condenser. Thermometers at the inlet and outlet of the condenser made it possible to record both of the temperatures.

A run was made using a bakers' pressed yeast slurry. The procedure was identical to the last experiment with the exception of the condenser. Samples were exploded at several temperatures, with and without the condenser. The results are recorded in Table XIV. The data, as plotted

Table XIV

Effect of precooling in continuous yeast explosion

Sample no.	T_1 [a]	T_2 [b]	Heating Time	Cooling time in min.	Per cent nitrogen in explodate	Odor
11	100	48	1.77	0.12	16.75	yeasty
12	120	55	2.22	0.14	20.0	yeasty
13	140	62	2.24	0.14	29.7	yeasty
14	160	68	2.28	0.14	42.2	burned
15	100	-	1.85	-	17.25	yeasty
16	120	-	0.97	-	18.7	yeasty
17	140	-	1.77	-	28.3	yeasty
18	160	-	1.83	-	43.5	burned

[a] Temperature of yeast at heat exchanger outlet.

[b] Temperature of yeast at condenser outlet.

in Figure 5, indicate that the cells, when heated and then cooled below 100° C. had approximately the same amount of nitrogen released as the cells that had not been cooled. This could be interpreted to mean, since the heating times are very similar, that temperature of cooking is a more important factor than internal pressure release in the liberation of nitrogen from the yeast cells. Furthermore, alteration of the cell walls by hydrolysis could be expected to increase directly with the temperature at the natural pH of 5 which was used. These altered cells may have some of the nitrogen released by mechanical action as they pass through the orifice.

Inskeep, et al. (107) explained that the primary centrifuges in a sulfite spent liquor yeast plant, concentrate the yeast cream from 1 per cent solids to 10 to 12 per cent solids. Subsequent washing produces a yeast cream of 14 to 18 per cent solids which is finally dried on drum driers. It was desired to see whether torula yeast could be exploded at relatively high concentrations, in order to take advantage of the existing yeast production facilities. One advantage to continuous explosion of high-concentration yeast slurries, is the saving realized on the heat required, assuming, of course, that the desired effect can be achieved at high concentrations.

In order to determine the exploding characteristics of torula yeast at relatively high concentrations, a 1450 g. sample of frozen, triple-washed, torula yeast was suspended in enough tap water to make 8 l. final volume. This was placed in the continuous, steam-heated exploder and exploded as indicated in Table XV. This slurry contained

Figure 5. Comparison of nitrogen released from yeast by heating effect and nitrogen released by combined heating and exploding effect

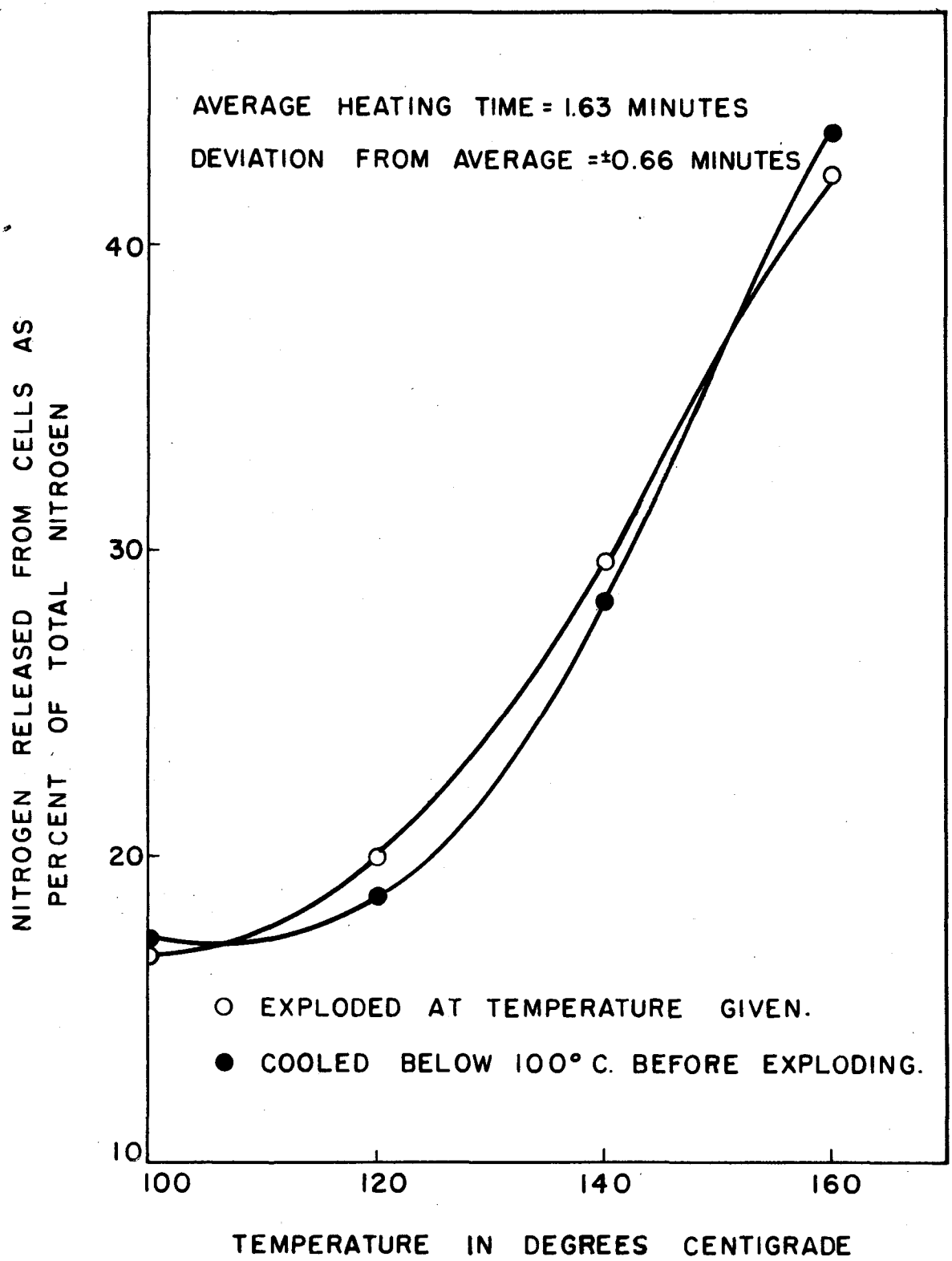

AVERAGE HEATING TIME = 1.63 MINUTES
DEVIATION FROM AVERAGE = ±0.66 MINUTES
NITROGEN RELEASED FROM CELLS AS PERCENT OF TOTAL NITROGEN
40
30
20
10
100
120
140
160
TEMPERATURE IN DEGREES CENTIGRADE
○ EXPLODED AT TEMPERATURE GIVEN.
● COOLED BELOW 100° C. BEFORE EXPLODING.

Table XV

Continuous explosion of high-concentration torula

Sample no.	Temperature ° C.	Heating time in min.	Per cent nitrogen in explodate
32	126	1.31	12.6
33	105	0.72	11.7

13.5 w/v per cent yeast solids. No difficulty was experienced in exploding the 13.5 per cent yeast slurry. A slurry of greater yeast concentration could have been handled easily by the heat exchanger. It is interesting to note that less nitrogen was exploded from the torula yeast cream of high concentration than was exploded from bakers' yeast samples of lesser concentration, i.e., as shown in Tables XIII, XIV, and XV. The advantages of this will be explained in the section entitled "Explosion of yeast followed by ethanol treatment."

4. Correlation and interpretation of yeast exploding information

The investigation of suitable processes for exploding yeast was initiated in the hope that this method for breaking cells would make it possible for all of the protein to be extracted from the yeast cell without the necessity of introducing acids, bases, salts, or other chemicals which would add to the expense and might prove deleterious to the final protein product. Theoretically, it should be possible to heat a yeast slurry in a closed system to a temperature higher than the

boiling point of the cell liquids at atmospheric pressure. Then, by releasing the pressure in the closed system, and consequently the external pressure on the yeast cells, more rapidly than the internal cell pressure can diffuse through the cell wall, a pressure differential can be created. It should be possible to heat the yeast cell liquids hot enough to provide a pressure differential great enough to break the cell wall. It has been the purpose of this investigation to see if the temperature necessary to explode the cells in this manner is in a practical range to be useful for the preparation of protein concentrates from yeast.

It has been found that it will not be possible to remove all of the protein from the yeast cells in a form suitable for human-grade protein concentrates using the conditions imposed in this investigation. The data graphed in Figure 5 rather conclusively point out that in the temperature range of 100° to 160° C., the amount of nitrogen released by the exploding effect is negligible compared to that liberated by the heating effect. Furthermore, according to Tables XII, XIII, and XIV, it will be impractical to use exploding temperatures above 160° to 200° C. unless the heating time is very short because high temperatures for long durations tend to produce a burned taste which would be very undesirable for producing human-grade protein concentrates from yeast.

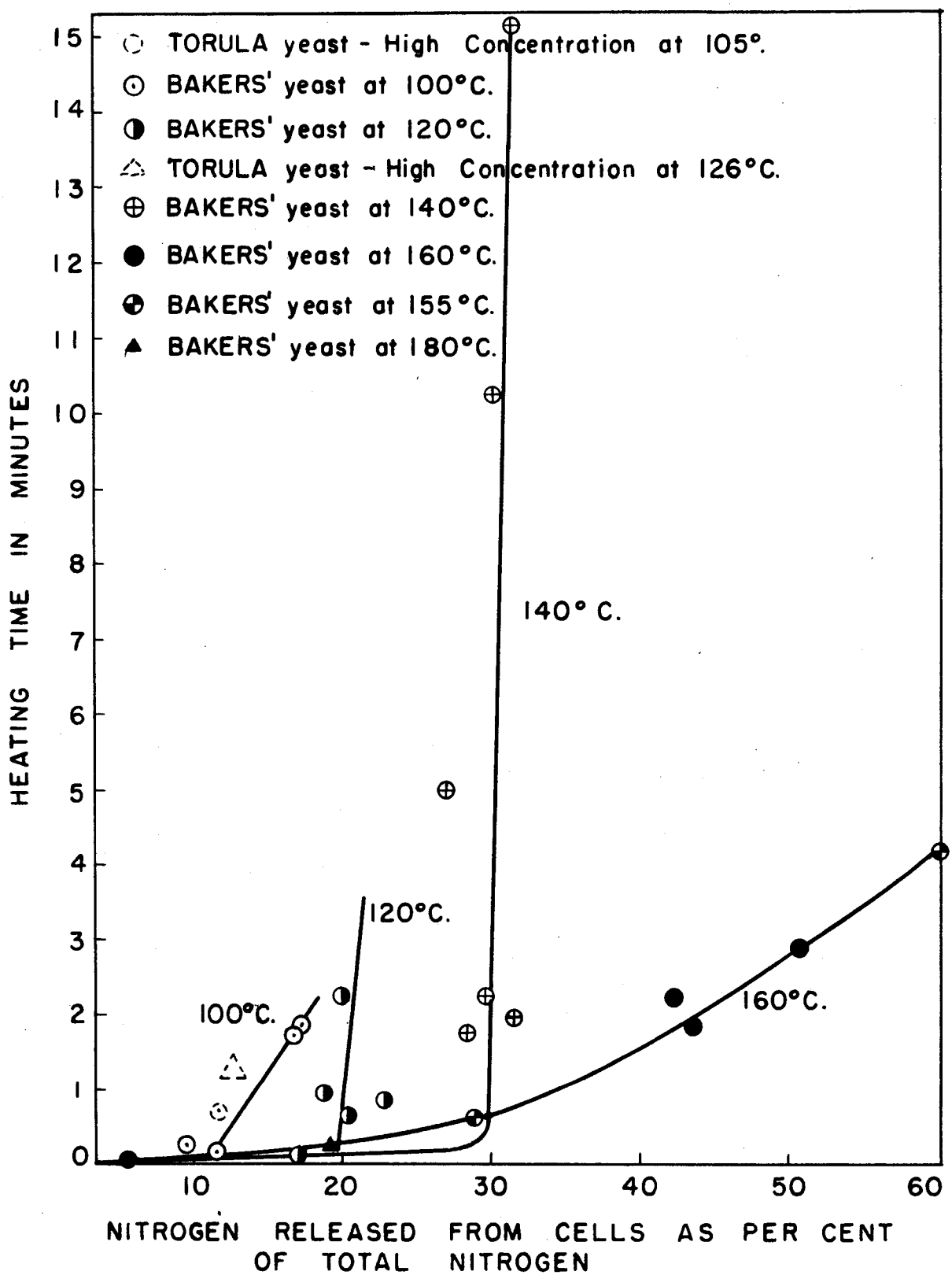
The liberation of nitrogen from the cells in these exploding experiments, while not due primarily to the exploding effect, is probably due to several heat-induced reactions. There is, of course, the possibility of decomposition of heat labile compounds in the cell.

Decomposition of cell wall compounds would change the permeability in proportion to the number of sites decomposed. Decomposition of cell cytoplasm would increase the number of molecules in the cell fluids. The change in osmotic pressure due to this effect might cause some osmolysis of the cell walls. An increase in destruction would be expected with an increase in temperature.

The release of nitrogen from the cells may be due to hydrolytic action at the high temperatures used in this investigation. The natural pH of 5.0 should be acidic enough to cleave peptide and polysaccharide bonds at high temperatures. According to Hall (90), an increase in the amount of hydrolysis is expected with increased heating time. The data recorded in Table VIII indicate that at 160 p.s.i. (188° C.) there is a 16 per cent increase in the amount of nitrogen liberated with a 5-minute increase in heating time. Figure 6, a graph of heating time versus per cent nitrogen released from the cells, correlates the data from Tables XII, XIII, XIV, and XV. This graph points out that at 160° C. there is an increased nitrogen release from the cells with increased heating time. However, for 140° C. this doesn't seem to hold true. This may possibly be explained as follows.

Hall (90) states that efficient protein hydrolysis requires an excess of acid to catalyze hydrolysis above that amount needed to form the acid salts of all the amino acids present. Water alone is not a sufficient hydrolysis catalyst because temperatures high enough to produce efficient hydrolysis are also high enough to cause protein destruction. His experiments show that temperatures above 160°

Figure 6. Comparison of the amount of nitrogen released from yeast at different temperatures and heating times


TORULA yeast - High Concentration at 105°.
BAKERS' yeast at 100°C.
BAKERS' yeast at 120°C.
TORULA yeast - High Concentration at 126°C.
BAKERS' yeast at 140°C.
BAKERS' yeast at 160°C.
BAKERS' yeast at 155°C.
BAKERS' yeast at 180°C.
HEATING TIME IN MINUTES
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
140° C.
120°C.
100°C.
160°C.
10
20
30
40
50
60
NITROGEN RELEASED FROM CELLS AS PER CENT OF TOTAL NITROGEN

to 162° C. cause protein destruction. The results of this investigation tend to bear out Hall's work. In almost every instance, as shown in Tables XII, XIII, and XIV, scorching, indicative of protein destruction, occurred when temperatures above 160° C. were used.

Thus, it may be seen that at 160° C. there is a continual destruction of protein with time. This is shown by the increase in the amount of nitrogen released from the cells. Proof that this nitrogen release is protein destruction rather than protein release from the cells is given in Table VIII and Figure 6. Here it is shown that there is no increase in the amount of alcohol-precipitable protein nitrogen, even though the amount of nitrogen released from the cells does increase with temperature and heating time.

At 140° C., as shown in Figure 6, there was not an increase of nitrogen release with increased heating time. The fact that 30 per cent of the nitrogen was released indicates that there was an alteration in the cell-wall permeability. It was not possible to extract the remainder of the nitrogen from the cell residues with water or 0.95 per cent saline. This would indicate that the permeability of the cell wall had not been sufficiently altered to allow unhydrolyzed proteins to pass through it. The 30 per cent nitrogen which was released from the cells represents the nitrogen already present in the cells as small molecules and that which was hydrolyzed from the existing complex protein molecules. Since very little nitrogen was being released by heat destruction of the proteins at 140° C. and water is a poor

hydrolysis catalyst at that temperature, the 30 per cent nitrogen release limit was reached because all of the acid or other radicals available for catalyzing hydrolysis had been inactivated by being combined with the amino acids or other compounds which were released in the process.

The protein which was released from the cells was in the form of small protein molecules. This was indicated in the procedure section as being due to the fact that the protein could be precipitated by ethanol but only formed a colloidal suspension with trichloracetic acid, a well known protein precipitant. The release of small molecules would seem to indicate a limited permeability alteration of the cell wall or that only a small percentage of the cells was being completely ruptured.

B. Removing Taste from Yeast Proteins

1. Extraction with fat solvents

From the work of Brasch (30), who extracted part of the taste from yeast with Skellysolve B, and Thomsen (252), who extracted the taste with hot vegetable oil, it was assumed that at least a part of the taste in yeast was due to the lipid fraction. Since 65 per cent of the nitrogen could be exploded and obtained cell free, an attempt was made to extract the lipids from the explodate with fat solvents in the hope of obtaining a tasteless yeast protein. Consequently, a 160 p.s.i. torula explodate was prepared in the quick-opening autoclave. The cell debris was removed by using a Sharples supercentrifuge followed by

filtration through a Hormann bacterial filter. The resultant explodate was extracted with a number of fat solvents to try and remove the lipids. This was done in a separatory funnel. Ethyl ether, hexane, Skellysolve B, carbon tetrachloride and Skellysolve D were all used. In every case, a stable foam and emulsion were formed which did not break down even after standing several days. It is possible that the carbon tetrachloride yeast protein emulsion might be used as a foam for fighting fires. However, the foam makes liquid-liquid extraction of the yeast proteins very difficult or impossible.

In order to test the antifoam properties of n-butanol, the following solvent mixtures were used to extract the explodate in a separatory funnel: 0.1, 1.0, 5.0 and 10.0 per cent butanol in hexane. Butanol inhibited the formation of the emulsion to a certain extent, but vigorous agitation created an emulsion which was just as stable as the hexane without butanol.

The above solvent extracted explodates were evaporated to dryness in an air stream on a low temperature steam bath. In all cases, the dried products resembled glue and tasted like paint so no attempt was made to analyze them.

A glass, liquid-liquid extractor was used to extract 200 ml. of 160 p.s.i., cell-free explodate with hexane. A small amount of stable emulsion was formed in the 24-hour extraction. Half of the extracted explodate was dried in an air stream over a steam bath. The dried product was again a dark brown, glue-like mass that tasted like paint.

The other half of the extracted explodate was dialyzed for 48 hours against tap water. The product was dried as before. It was quite tasteless when dry.

Unextracted, 160 p.s.i. explodate was dialyzed 48 hours against tap water and then air dried on the steam bath. The dark brown solids were tasteless and odorless, but the yield was too small to be of any practical value.

A Soxhlet extractor was used to extract dry torula fodder yeast with hexane for 24 hours. One half of this product was made into a water slurry and dialyzed against tap water for 48 hours. The solids were filtered out and air dried at 70° C. The undialyzed solids were also air dried. There was little difference in taste between the original untreated dry yeast, the hexane extracted and the extracted and dialyzed yeast. Hexane had very little effect on the taste of drum dried torula yeast.

It was concluded that the bulk of the taste-producing molecules were not hexane soluble but were small enough to be dialyzed away from the protein molecules. Since dialysis is a rather slow process for continuous commercial production of protein concentrates, further investigations of this procedure were discontinued.

2. Extraction with vegetable oil

Thomsen (252) claimed that vegetable oil heated to 100° C. would remove the taste from yeast products. In order to test his theory, a

sample of bakers' pressed yeast was extracted with Wesson oil. A 300-g. sample of yeast was dispersed in 1 l. of Wesson oil in a Waring blender. This rather viscous slurry was heated in a 2-liter beaker on a hot plate. At a temperature of 40° C., the yeast began coagulating and formed a peanut butter-like lump on the stirring rod. At 60° C., the yeast dispersed again and became like thin brown mud at the bottom of the beaker. At 80° C. the yeast formed small paste-like crumbs dispersed throughout the beaker, and at 100° C. the moisture in the cells began to boil off and a slight amount of foam formed. A time interval of 15 minutes was required to bring the mixture to a boil. It was allowed to boil (100° C.) for 5 minutes more and then the beaker was placed in cold water to cool.

The yeast sludge, when centrifuged, was still too oily to be useful. Consequently, the oil from 15 ml. of the yeast sludge was extracted with acetone. This did a good job of removing the oil and left a light brown product with a dry crumby texture. When air dried at 50° C., this material had a bad taste. It was not a typical yeast taste, however.

A 10-ml. aliquot of the oily yeast sludge was extracted with n-hexane in a beaker. There was enough residual moisture in the cells to make the yeast pasty in the presence of the hexane. As a consequence, it was impossible to extract all of the oil with the cold hexane. The partially extracted material, when dry, had both a yeasty taste and an oily taste. The partially extracted material, when acetone extracted and dried, had the same taste as that which was extracted with acetone alone.

It was possible to remove the oil from the yeast by extracting it for 24 hours with hexane in a Soxhlet extractor. However, when dry the product still had a strong yeast taste.

3. Extraction and precipitation with ethanol

From the previous experiments, it was found that fat solvents did not remove all of the yeast taste. It was, therefore, concluded that some of the yeast taste might be due to the small water or alcohol soluble molecules which are associated with the protein. The removal of these molecules from the protein would include the removal of the B vitamins, small peptides, amino acids and the like. In this investigation, the word "detaste" was used to indicate the removal of the taste-producing small molecules from the protein.

It is a well recognized fact that ethanol can be used for a protein precipitant (9, 20, 140). Ethanol, however, has not been used to produce bland yeast proteins commercially. The reason given (30) is that the B vitamins would be extracted. A dry, bland yeast protein without the vitamins, however, could be very useful in nutrition if its limitations were well recognized.

In order to test the effect of 95 per cent alcohol on live yeast, a 25-g. sample of pressed bakers' yeast was extracted with 150 ml. of cold 95 per cent ethanol for 30 minutes in a Waring blender. The yeast slurry was filtered and the filter cake crumbled and air dried at 70° C. The product was a creamy-white powder that maintained most, but not all, of the typical yeast taste. It was concluded that cold alcohol does not

have the ability to alter the permeability of the cell wall enough to extract all of the taste-producing molecules from live yeast. It can be assumed, of course, that if sufficient time were allowed, cell alteration could have been effected by autolysis, since ethanol is an antiseptic used for autolysis.

It was thought that hot ethanol might alter the cell wall permeability enough to extract any alcohol soluble molecules. With this in mind, 100 g. of pressed bakers' yeast and 250 ml. of ethanol were placed in a beaker over a hot plate. The initial temperature was 28° C. It required 8 minutes to reach the boiling point. The yeast was immediately filtered and the filter cake crumbled and air dried at 70° C. This product was a creamy-brown powder and still retained most of the yeast flavor.

Another experiment was performed to see if prolonged boiling with ethanol would remove the flavor from yeast. A 25-g. sample of bakers' yeast was heated with 150 ml. of ethanol. It required 7 minutes to heat the yeast slurry from 29° C. to the boiling point at 78° C. The yeast-alcohol slurry was then boiled for 5 minutes additional and then quickly filtered and air dried at 70° C. The taste, color and texture were identical with the sample which did not have the prolonged boiling. The color was slightly darker than the cold ethanol extracted yeast, but the flavor was about the same.

A 25-g. sample of dry torula fodder yeast was extracted with 150 ml. of ethanol. The mixture was heated on a hot plate and

continuously agitated. It required 5 minutes for the sample to be heated from 28° to 78° C. The sample was immediately filtered and air dried at 70° C. The taste was not altered appreciably from that of the unextracted original.

Approximately 150 ml. of boiling water were poured on 25 g. of pressed bakers' yeast in a beaker. The temperature immediately fell to 85° C. The slurry was heated on a hot plate with constant stirring. It required 3 minutes to raise the temperature to 100° C. The yeast slurry was boiled for 2 minutes more. This slurry was then cooled by setting the beaker in cold water. The nitrogen analysis of the homogeneous material and of a cell free sample indicated that 17.7 per cent of the total nitrogen was liberated from the cells. This falls in the same range of nitrogen liberation as was found in the exploding experiments shown in Figure 6.

The remainder of the yeast slurry was treated with twice its volume of cold ethanol to precipitate the protein. This was filtered and the protein cake air dried. It was a light grey-brown in color, and it retained a fairly strong typical yeast flavor. However, it did not have so strong a flavor as unextracted dry yeast.

4. Explosion of yeast followed by ethanol treatment

The original purpose of the yeast exploding experiments was to extract all of the yeast proteins from the cells so that the taste-producing small molecules might be removed from them. In order to test

this theory, 400 g. of bakers' pressed yeast was suspended in enough water to make 700 ml. This was exploded at 160 p.s.i. steam pressure by being released from the draincock orifice of the quick-opening, batch autoclave.

A 200 ml. sample of the homogeneous exploded material was centrifuged for 15 minutes at 2000 r.p.m. to remove the cell debris. The debris, containing 35.5 per cent of the total nitrogen, was discarded and the supernatant treated with 2 times its volume of 95 per cent ethanol to precipitate the protein. The protein was recovered by centrifuging. It was then dried at 70° C. in a vacuum oven. The pure protein tends to form a glue-like mass which is very difficult to grind when dry. However, it is practically tasteless even as a fine powder. The only taste is a slightly scorched taste.

A 400 ml. sample of the above exploded yeast homogenate was treated with twice its volume of ethanol. This precipitated both cell residue and protein. When vacuum dried at 70° C., this dark colored product was practically tasteless. Again the scorched taste was predominant.

The extraction of live yeast cells with hot and cold ethanol clearly showed that the cells needed a pre-treatment before ethanol would be effective in extracting the taste-producing small molecules. It was found in the preceding experiment that all but the scorched taste could be removed from the cell residue by alcohol precipitation. Therefore, the emphasis of attack was changed from trying to get the

most protein exploded out of the cells to one of leaving the most protein in the cells and then removing the taste with ethanol. Further necessity of this may be seen in Tables XI, XII, and XIII, which point out that 89 to 150 p.s.i. or 160° to 185° C. are the upper temperature limits which can be used without scorching the yeast under the conditions used in this investigation. At temperatures below this, less than half of the yeast nitrogen is exploded from the cell. Thus, moderate heating temperatures should be used in order to keep as much nitrogen as possible in the cells. This will be regulated by the amount of heat necessary to alter the permeability of the cell wall enough for small molecule extraction.

To test this new approach, 2800 ml. of sample No. 3, shown in Table XIII, was used. This was a bakers' yeast slurry which was exploded at 120° C. with a heating time of 0.875 minutes in the steam-heated, continuous exploder. A total of 22.7 per cent of the nitrogen was exploded from the yeast. Part of this explodate nitrogen, 9.83 per cent of the total nitrogen, was alcohol precipitable.

An 1800 ml. aliquot of sample 3 was treated with twice its volume of cold ethanol. Approximately 13.6 per cent of the total nitrogen remained in the alcohol supernatant. The yeast residue was centrifuged and the residue dried at 70° C. This dried to a hard, brown, crumby material which was practically tasteless. Apparently, centrifuging leaves too much moisture in the sludge, and this adversely affects the protein during drying. The moisture causes the protein to set like

glue when the material is air dried slowly in the laboratory. This could probably be eliminated by rapid commercial spray drying.

A 925-ml. aliquot of sample 3 was centrifuged and the clear golden supernatant decanted. The cell residue was divided into two parts. One part was washed with water, centrifuged and air dried. The dark brown glue-like solid still retained the yeast taste when ground with a mortar and pestle. The other half of the residue was washed with 100 ml. of cold ethanol, centrifuged and the precipitate dried. This dried to brown, hard crumbs which were nearly tasteless when powdered.

The centrifugate from the above aliquot of sample 3 was treated with 2 volumes of cold ethanol to precipitate the protein. This protein was separated by centrifuging and air dried at 70° C. The product was light grey when powdered and had a nut-like flavor. As shown in Table XIII, this was only 9.83 per cent of the total protein in the yeast.

Sample 15, shown in Table XIV, was exploded in the steam-heated, continuous exploder at 100° C. The heating time was 1.85 minutes and 17.25 per cent of the nitrogen was released into the explodate. A 200-ml. aliquot of sample 15 was centrifuged, and the cell debris was washed once with water. Following this, it was centrifuged and air dried at 70° C. This product (labeled 15a) dried to hard glue-like particles which powdered with difficulty and had the typical yeast flavor.

A 500-ml. aliquot of sample 15 was treated with twice its volume of cold ethanol. The cells and precipitated protein were filtered on a Buchner funnel and the relatively dry cake crumbled and passed through a No. 16 seive (0.0469 in. interstices). The sample was then air dried at 70° C. The product was a light-cream colored powder. There was no tendency for it to set like glue upon drying. It had a typical yeast taste, but this taste was not so strong as untreated dried yeast. The flavor and intensity of the flavor were similar to those obtained by boiling yeast with water in an open beaker and then precipitating the cells with ethanol as previously described.

Sample 16, shown in Table XIV, was a bakers' pressed yeast slurry exploded in the steam-heated exploder at 120° C. The heating time was 0.97 minutes and 18.7 per cent of the protein was released into the explodate.

A 1000-ml. aliquot of sample 16 was centrifuged and the precipitate washed with 500 ml. of water and again centrifuged. The centrifugates were combined and treated with 2 volumes of cold ethanol. The precipitated protein was filtered on a Buchner funnel and air dried at 50° C. The product, representing less than 10 per cent of the original yeast protein, was brown and had a very slight nut-like taste.

The washed precipitate of sample 16, mentioned above, was divided into four equal parts. The first part was air dried to a glue-like mass at 70° C. When powdered, it had a typical yeast taste but not quite so strong as air dried, pressed bakers' yeast.

The second part of the precipitate was treated with 200 ml. of acetone, filtered, crumbled and air dried at 70° C. The product was a nice light-cream colored powder with a horrible taste.

The third part of the precipitate was treated with 200 ml. of Skellysolve B. The solvent was relatively immiscible with the wet yeast paste. When air dried at 70° C., it formed hard glue-like particles which when powdered still retained the yeast taste.

The fourth part of the precipitate was treated with 200 ml. of cold 95 per cent ethanol. The residue was filtered, the cake crumbled and air dried at 70° C. The product was light-cream colored and was very bland. It did not have the typical yeast taste but did have a slight pleasant after-taste described as nut-like by some of the taste panel. One Chinese student described the taste as being similar to popped rice and quite acceptable. The dry product contained 57 per cent protein.

A 1000-ml. aliquot of the homogeneous sample 16, exploded as described in Table XIII, was treated with twice its volume of cold ethanol. This precipitated both cells and protein. The residue was filtered, crumbled and air dried at 70° C. The product was a light-cream color and had a very bland taste similar to, but slightly stronger than, the alcohol washed cell residue described above. This dry product contained 59.4 per cent protein (nitrogen x 6.25).

Table XV gives the experimental data concerning the exploding of torula yeast sample 32. A heating time of 1.31 minutes at 126° C. was used and 12.6 per cent of the nitrogen was released into the explodate.

A 1 l. aliquot of sample 32 was centrifuged. This aliquot contained 13.4 g. of total nitrogen. The cell debris was washed three times with 500 ml. of ethanol, then filtered and air dried at 50° C. A bland, creamy-white powder, 32a, was obtained which weighed 108 g. It had the typical yeast taste but very slight compared with the original yeast. It contained 59 per cent protein (nitrogen x 6.25). The total nitrogen recovered in product 32a was 10.2 g. or 76.5 per cent of the total nitrogen in the homogenate. The analysis of nitrogen released from the cells as described in the procedure section, indicated that 87.4 per cent of the nitrogen remained in the cell debris after centrifugation at 6000 r.p.m. The difference of 10.9 per cent between the amount left in the cells at 6000 r.p.m. and the actual recovery may be attributed to two causes: 1, the solubility of some of the nitrogen compounds left in the exploded cells; 2, the poor recovery of cell material due to the inefficient centrifugation of the larger batch at 2000 r.p.m.

The yeast taste in product 32a, although not very strong, could be completely removed by the following treatment which left only a slight nut-like taste. A 38-g. sample of dry product 32a was extracted with 500 ml. quantities of solvents by suspension for 5 minutes each in a Waring blender, followed each time by filtration. The sequence of solvents is as follows:

1. One part hexane and three parts ethanol.
2. One part hexane and three parts ethanol.
3. Ethanol.
4. Ethanol.

The final product, 32b, was filtered, crumbled and air dried to a fluffy, cream-colored powder. This was the blandest product prepared in this investigation. Approximately 98.7 per cent of the weight of the starting material was recovered.

A 1 l. aliquot of exploded homogenate of sample 32 was treated with 2 volumes of ethanol. Protein and cell debris were precipitated and filtered. The cake was washed in the Waring blender with 500 ml. of ethanol and again filtered. The filter cake was crumbled and air dried at 50° C. This product, 32c, was a cream-colored powder with a yeasty taste stronger than product 32a.

Sample 33 was described in Table XV. It was a torula yeast cream containing 13.5 per cent solids. It was exploded at 105° C. with a heating time of 0.72 minutes. Analysis revealed that 11.7 per cent of the nitrogen was released from the cells by exploding.

Aliquots of sample 33 were given the same treatment as products 32a and 32c just described above. The respective products could be rated as to intensity of yeast taste as follows: 32b < 32a < 32c < 33a < 33b < untreated yeast.

The detasting data indicate that, if the yeast cell membranes have been made sufficiently permeable, it is possible to separate the small, taste-producing molecules from the protein and produce a bland protein concentrate. It is also evident that the degree of yeast taste removal is dependent upon the completeness of extraction. The most successful

extractions were performed on yeast cells exploded at 120° to 126° C. with a total heating time less than 3 minutes.

C. Spectrographic Analysis of Torulopsis utilis

Bandt (16) investigated the suitability of torula yeast for fish food. He used torula grown on European spent sulfite liquor, and found evidence of toxicity which he attributed to detectable amounts of lead, copper and arsenic salts found in the yeast.

In order to check the safety of American torula yeast for use as food, dry torula fodder yeast was analyzed for toxic elements which might have been obtained from the spent sulfite liquor medium upon which they were grown. The yeast that was used contained 8.8 per cent moisture. The yeast was ashed to prepare it for spectrographic analysis. Sample No. 1 was ashed plain. Sample No. 2 was a sulfate ash. It was prepared by treating the yeast sample with a few drops of concentrated nitric acid and two or three drops of sulfuric acid. The samples were then ignited to constant weight in porcelain crucibles.

A complete qualitative spectrographic analysis was performed by the Ames Laboratory of Iowa State College. The results are shown in Table XVI. This analysis indicates that no lead or arsenic are present and only a small amount of copper is present.

D. Nitrogen Released by Yeast Autolysis

This experiment was designed to find the optimum time for nitrogen release from bakers' yeast during autolysis. It was also desired to find

Table XVI

Spectrographic qualitative analysis of torula yeast

Element	Plain ash[a]	Sulfate ash[a]
Silver	VW	VW
Aluminum	VW	VW
Boron	FT	T
Barium	T	T
Calcium	S	S
Chromium	VFT	VFT
Copper	VW	VW
Iron	M	M
Potassium	VS	VS
Lithium	W	W
Magnesium	S	S
Manganese	W	W
Sodium	S	S
Phosphorous	M	M
Silicon	VW	VW
Tin	VFT	VW
Zinc	T	T

[a]The symbols have the following significance: V = very, S = strong, M = moderate, W = weak, F = faint, T = trace.

the amount of protein nitrogen released from the cells. It was hoped that this information would be useful in determining the suitability of autolysis as a cell-rupturing step in the production of bland proteins.

Farrer (65, 66), in determining optimum conditions for the autolytic release of some of the B vitamins, found that 50° to 52° C. was the optimum autolysis temperature. He reported that the optimum pH was the natural pH of yeast in water, which is approximately 5.0.

The yeast used in this experiment was bakers' pressed yeast which was washed three times with ice cold physiological saline. The washed yeast was suspended in physiological saline, making 500 ml. of slurry containing approximately 10 per cent solids. This yeast slurry, which had a pH of 5.0 was placed in a 500 ml. two-neck, round bottom flask which was fitted with a leak-tight stirrer with well-lubricated, ground glass bearing surfaces.

The nitrogen liberated by autolysis was determined by periodically removing a 10-ml. sample from the autolysis flask and transferring it to a 15-ml. centrifuge tube. This sample was cooled for 18 seconds in a dry ice-acetone bath. This reduced the temperature of the autolyzate from 50° to 4° or 5° C. This cooling was thought to be necessary in order to minimize the enzymatic degradation of the protein in the autolysate prior to protein precipitation. The chilled sample was then centrifuged for 15 minutes at approximately 3000 r.p.m.

The centrifugate contained only saline-soluble nitrogen compounds. The centrifugate was transferred to a 25-ml. volumetric flask, and the precipitate was washed with ice cold saline and again centrifuged. The centrifugates were combined and placed in a 20° C. water bath to reach calibration temperature. The centrifugates were made up to 25 ml. and the Kjeldahl-nitrogen samples removed.

In order to determine the amount of protein nitrogen released during autolysis, an aliquot of the combined centrifugates, prepared as described above, was mixed with an equal volume of 10 per cent trichloracetic acid. This was allowed to stand for at least 10 minutes to precipitate the protein and then it was centrifuged. The centrifugate was discarded and the protein precipitate thoroughly drained. The protein was dissolved in water or 3 per cent sodium hydroxide and made up to 5 ml. volume for Kjeldahl-nitrogen analysis.

Table XVII tabulates the results of this experiment. Figure 7, a graph of autolysis time versus per cent of nitrogen in autolysate, indicates that there is a rapid nitrogen release up to about 77.2 per cent at 500 minutes, and then the rate of liberation levels off and only 89.6 per cent of the nitrogen is liberated at 4,120 minutes. It is apparent from Table XVII that the amount of protein nitrogen liberated is negligible compared to the total nitrogen present in the yeast. This would minimize the possibility of using the released nitrogen for the preparation of bland proteins. However, a relatively short autolysis time might alter the cell wall sufficiently to allow extraction of the taste-producing small molecules from the protein with alcohol. This was not done in this investigation.

Table XVII

Nitrogen released by yeast autolysis

Sample No.	Time in min.[a]	Per cent nitrogen[b] in autolysate	Per cent nitrogen[b] as protein nitrogen in autolysate
1	10	0.0	0.0
2	30	19.8	0.078
3	50	23.2	0.44
4	70	27.1	0.32
5	100	32.2	0.97
6	130	37.2	0.61
7	160	43.2	0.365
8	190	43.0	0.078
9	220	50.4	-
10	250	54.5	0.047
11	280	64.8	0.00
12	340	68.5	
13	410	75.5	
14	520	77.2	
15	640	80.0	
16	760	83.1	
17	1120	84.5	
18	1480	87.0	
19	1790	88.2	
20	2730	88.9	
21	3235	89.0	
22	4120	89.6	

[a]Time was determined from the time the yeast slurry reached 50° C. It required 10 minutes to heat the slurry from 10° C. to 50° C.

[b]The nitrogen per cent was based on the total nitrogen in the slurry.

Figure 7. The total nitrogen and protein nitrogen released from bakers' yeast autolyzed at 50° C.

AUTOLYSATE NITROGEN AS PERCENT OF TOTAL N.
0
10
20
30
40
50
60
70
80
90
100
AUTOLYSIS TIME IN MINUTES
0
200
400
600
800
1000
1200
1500
AUTOLYSATE NITROGEN
PROTEIN NITROGEN
PROTEIN NITROGEN IN AUTOLYSATE AS PERCENT OF TOTAL N.
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

E. Isolation and Nutritional Requirements of Cellulolytic Rumen Bacteria

Work by Kitts (124), Quinn (195), and Ruf (215) has indicated that there is a factor(s) in yeast extracts which promotes cellulose digestion by cellulolytic bacteria. A part of this investigation was devoted to the development of media which would make it easier to isolate cellulolytic organisms and make it possible to analyze for the unknown cellulose digestion factor using transparent media with CMC-70L as the cellulose source. CMC-70L is a sodium salt of one of the derivatives of carboxymethylcellulose. It has relatively few carboxymethyl substitutions per glucose residue (94). It dissolves readily in water in low concentrations and forms a water clear solution. A part of this investigation was also devoted to classifying some of the characteristics of the unknown factor(s).

1. Isolation

This work was initiated cooperatively with Dr. Kitts, by isolating 32 colonies of rumen organisms. In his thesis (124) he carefully described the procedure used in diluting the rumen liquor and inoculating it into tubes of rumen agar and Eugon agar. From these agar tubes 33 individual colonies were picked and transferred to a trypticase-phytone medium (medium No. 1). Only one of the colonies failed to grow.

Since many different bacteria besides cellulolytic bacteria grow in the various media which were used to isolate these colonies, it was desirable to isolate the cellulose digesters and determine some of their

fermentation characteristics. Consequently, each of the 32 organisms was used to inoculate (see procedure) duplicate 15 x 125 mm. tubes of each of the sterile, anaerobic media described below. Each tube contained 6 ml. of medium. The fermentation characteristics of the isolates are tabulated in Table XVIII, which follows the description of the media.

In each of the media given below, solutions A, B, and C had the following composition per 20 ml.

Mineral solution A

Ammonium sulfate	0.05 g.
Potassium phosphate.$3H_2O$ (dibasic)	0.05 g.
Potassium phosphate (monobasic)	0.02 g.
Calcium chloride	0.005 g.
Magnesium sulfate	0.005 g.
Sodium chloride	0.10 g.

Solution B

Sodium thioglycolla te	0.05 g.
Cysteine hydrochloride	0.02 g.
Cystine	0.075 g.
Glucose	0.20 g.
Cellobiose	0.20 g.
Sodium bicarbonate	0.25 g.
Sodium carbonate	0.30 g.

Solution C

Sodium thioglycollate	0.05 g.
Cysteine hydrochloride	0.02 g.
Cystine	0.075 g.
Sodium carbonate	0.3 g.
Sodium bicarbonate	0.25 g.

Resazurin dye was used in some of the media as a redox indicator. It made the media light orange when it was anaerobic and light pink when exposed to trace amounts of oxygen.

The CMC-70L dissolved easily by making a concentrate in water containing 5 g. per 100 ml. of solution and agitating it in a Waring blender. Only 20 ml. of this concentrate is necessary to supply 1 g. per 100 ml. of medium.

Medium No. 1. Trypticase - phytone

Trypticase		1.5 g.
Phytone		0.5 g.
Sodium chloride		0.4 g.
Sodium citrate		0.1 g.
Sodium sulfite		0.02 g.
L-Cystine		0.02 g.
Glucose		0.5 g.
Mineral solution A		20.0 ml.
Solution B		20.0 ml.
Distilled water	to	100.0 ml.

Medium No. 2. Eugon broth

Medium No. 2 was a broth medium adapted from the work of Deutsch and coworkers (57). The Eugon broth had the same composition as Eugon agar minus the agar (see Materials).

Eugon broth powder		3.04 g.
Mineral solution A		20.0 ml.
Solution B		20.0 ml.
Resazurin dye		0.0001 g.
Distilled water	to	100.0 ml.

Medium No. 3. Yeast extract

Bacto yeast extract		3.5 g.
Mineral solution A		20.0 ml.
Solution B		20.0 ml.
Resazurin dye		0.0001 g.
Distilled water	to	100.0 ml.

Medium No. 4. Rumen extract

Rumen extract		15.0	ml.
Mineral solution A		20.0	ml.
Solution B		20.0	ml.
Resazurin dye		0.0001	g.
Distilled water	to	100.0	ml.

The rumen extract was made by filtering rumen contents through cheese cloth and then autoclaving for 15 minutes at 15 p.s.i. The autoclaved material was then clarified by using first a Sharples supercentrifuge and then filtering the liquor through a Hormann bacterial filter.

Medium No. 5. Yeast Cellulose extract

This medium was adopted from the best medium reported by Quinn (195) and designated as No. D-58 in his thesis. The original cellulose prescribed was replaced by CMC-70L. The composition is as follows:

Bacto yeast extract		3.5	g.
Ammonium sulfate		1.0	g.
Calcium chloride		0.1	g.
CMC-70L		1.0	g.
Monobasic sodium phosphate.H_2O		1.3	g.
Dibasic sodium phosphate.$7H_2O$		6.44	g.
Magnesium sulfate.$6H_2O$		2.0	g.
Resazurin		0.01	g.
Distilled water	to	1.0	l.

Medium No. 6. Without carbohydrate

This medium is the same as Medium No. 5 without the CMC-70L.

Medium No. 7. Yeast extract - cellobiose

This medium is the same as Medium No. 5 with 1 g. of cellobiose replacing the CMC-70L.

Medium No. 8. Yeast extract - Alphacel

This medium is the same as Medium No. 5 with 1 g. of Alphacel cellulose replacing the CMC-70L.

Medium No. 9. Trypticase - without carbohydrate

Trypticase		2.35	g.
Mineral solution A		20	ml.
Solution B		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 10. Trypticase - CMC-70L

This medium is the same as Medium No. 9 with the addition of 1 g. of CMC-70L.

Medium No. 11. Rumen liquor - CMC-70L

Rumen liquor		15	ml.
Phenol red		0.0002	g.
Mineral solution A		20	ml.
CMC-70L		1	g.
Solution C		20	ml.
Distilled water	to	100	ml.

Medium No. 12. Rumen liquor - glucose

Rumen liquor		15	ml.
Glucose		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 13. Rumen liquor - maltose

Rumen liquor		15	ml.
Maltose		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 14. Rumen liquor - cellobiose

Rumen liquor		15	ml.
Cellobiose		0.05	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 15. Rumen liquor - solka floc

Rumen liquor		15	ml.
Solka floc (see Materials)		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 16. Rumen liquor - dextrin

Rumen liquor		15	ml.
Dextrin		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 17. Rumen liquor - starch

Rumen liquor		15	ml.
Starch (soluble)		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 18. Rumen liquor without carbohydrate

Rumen liquor		15	ml.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Medium No. 19. Yeast extract - CMC-70L

Bacto yeast extract		3.5	g.
CMC-70L		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Phenol red		0.0002	g.
Distilled water	to	100	ml.

Media 11 to 19 had a 6 x 30 mm. gas tube in each culture tube with the 6 ml. of medium. Each tube was inoculated with 0.5 ml. of culture (see Procedure). Medium No. 1 was used to grow the inoculum for media 2 to 10, and Medium No. 3 was used for growing the inoculum for media 11 to 19. The results tabulated in Table XVIII are for two organisms (designated as Nos. 31 and 32 in future experiments). They were the only organisms that actively fermented cellulose although all 32 organisms fermented starch, dextrin, maltose, cellobiose and glucose.

The cellulose digestion characteristics of bacteria 31 and 32 were described by Kitts (297). He also displayed electron photomicrographs of these bacteria. The bacteria in culture 31 were Gram positive non-motile cocci. They occurred singly and in short chains. The bacteria in culture 32 were Gram variable, motile rods. In young cultures, they were Gram negative and in older cultures they were Gram positive. Both cultures were strict anaerobes.

Culture No. 32 was the most vigorous; consequently, it was chosen as the only one to be used in further work on the cellulolytic growth factor. It grew well in Eugon agar tubes forming a characteristic disk-to biconvex lens shaped colony. The Eugon agar had the following composition.

Table XVIII

The reaction of two cellulolytic bacteria to various media

Medium No.[a]	Organism No. 31			Organism No. 32		
	Growth	Acid	Gas	Growth	Acid	Gas
1	4+			4+		
2	4+			4+		
3	2+			2+		
4	4+			4+		
5	b			b		
6	b			b		
7	b			b		
8	b			b		
9	N	N		N	N	
10	3+	Y		2+	Y	
11	3+	Y	N	4+	Y	N
12	2+	Y	N	-	-	-
13	3+	Y	N	4+	Y	N
14	3+	Y	N	3+	Y	Y
15	2+	Y	N	2+	N	N
16	4+	Y	N	4+	Y	Y
17	4+	Y	N	4+	Y	N
18	1+	Y	N	1+	Y	N
19	3+	Y	N	3+	Y	N

[a]The meaning of the symbols used is: Y, yes; N, no; b, Too much inorganic precipitate formed in these media to detect growth visually; Growth is denoted by t; and the numbers 1 through 4 denote relative increasing growth in that order.

Eugon agar (see Materials)		4.54	g.
Mineral solution A		20	ml.
Solution B		20	ml.
Resazurin dye		0.0001	g.
Distilled water	to	100	ml.

Each tube of sterile anaerobic Eugon agar was inoculated with 0.5 ml. of 10^{-6}, 10^{-7} or 10^{-9} dilution of pure cultures of organism No. 32 (see Procedure). The agar was gassed with carbon dioxide and stoppered. The tube was then placed in a horizontal position and rotated in order to solidify the agar in a film on the sides of the tube. These tubes were incubated 48 hours at 39° C. About two colonies per tube were observed in the tubes inoculated with the 10^{-9} dilution.

Since the Eugon Agar is a non-specific medium, it was desirable to have an agar medium which would contain only cellulose as the carbohydrate source. This would tend to minimize the possibility of contaminating organism 32 and provide an isolation agar for cellulolytic bacteria; consequently, a number of agar media were investigated. They were inoculated in the same manner as the Eugon agar just described above. The composition of these agar media is given below. In all these media, mineral solution K had the following composition for each 40 ml.

Mineral solution K

Calcium acetate		0.0	g.
Ammonium sulfite		0.02	g.
Magnesium chloride·$6H_2O$		0.01	g.
Potassium phosphate (monobasic)		0.02	g.
Sodium phosphate (dibasic)		0.05	g.
Sodium thioglycollate		0.05	g.
Urea		0.1	g.
Manganous chloride·$4H_2O$		0.00125	g.
Sodium tetraborate		0.0025	g.
Cobaltous chloride·$4H_2O$		0.0005	g.
Sodium carbonate		0.5	g.
Distilled water	to	40.0	ml.

Agar No. 1. Yeast extract - CMC-70L

Bacto yeast extract		1.0	g.
CMC-70L		1.0	g.
Mineral solution K		40.0	ml.
Agar		1.5	g.
Distilled water	to	100.0	ml.

Agar No. 2. Yeast extract - CMC-70L - Cellobiose

Bacto yeast extract		1.0	g.
CMC-70L		1.0	g.
Cellobiose		0.2	g.
Mineral solution K		40.0	ml.
Distilled water	to	100.0	ml.

Agar No. 3. Torula extract - CMC-70L

Torula yeast extract		20	ml.
CMC-70L		1	g.
Mineral solution K		40	ml.
Agar		1.5	g.
Distilled water	to	100	ml.

Agar No. 4. Torula extract - CMC-70L - Cellobiose

No. 4 was the same as No. 3 with the addition of 0.2 g. cellobiose.

The torula extract was made by mixing thoroughly 3 l. of boiling water with 600 g. of dry torula fodder yeast in a beaker. The solids were centrifuged out and the centrifugate was treated with 2 volumes of methanol to precipitate the protein. The resulting extract was evaporated to 1500 ml. to remove the methanol.

Duplicate tubes of isolation agars 1 through 4 were inoculated with 1 ml. of 10^{-6}, 10^{-7} or 10^{-9} dilution of organism 32 as previously described and incubated 48 hours at 39° C. They were all transparent

agars with a slight opalescence. They all tended to be too soft at the 39° C. incubation temperature.

Agar No. 5. Torula extract - CMC-70L

Torula yeast extract		20	g.
CMC-70L		1	g.
Mineral solution K		40	ml.
Agar		1.75	g.
Phenol red pH indicator		0.0005	g.
Distilled water	to	100	ml.

Agar No. 6. Yeast extract - CMC-70L

Bacto yeast extract		1	g.
CMC-70L		1	g.
Mineral solution K		40	ml.
Agar		1.75	g.
Resazurin		0.0001	g.
Distilled water	to	100	ml.

Agar No. 7. Torula extract - CMC-70L

Torula yeast extract		20	ml.
CMC-70L		1	g.
Mineral solution K		40	ml.
Agar		1.75	g.
Resazurin		0.0001	g.
Distilled water	to	100	ml.

Agar No. 8. Rumen liquor - CMC-70L

Rumen liquor (Seitz filtered)		20	ml.
CMC-70L		1	g.
Mineral Solution K		40	ml.
Ammonium molybolate		0.00005	g.
Resazurin		0.0001	g.
Agar		1.75	g.
Distilled water	to	100	ml.

Tubes of agars No. 5 through 7 were inoculated with 1 ml. of 10^{-6}, 10^{-7} or 10^{-9} dilution of fresh rumen liquor (see Procedure).

All of the agars 1 through 8 were transparent and no difference could be detected in their ability to support growth of organism No. 32. Discrete colonies appeared in the 10^{-9} dilution tubes. Colonies from organism 32 were disk and biconvex lens shaped. They were about 0.2 to 0.5 mm. in diameter at 48 hours, but on further incubation for 4 weeks attained a diameter of about 2 mm. It was relatively easy to pick these colonies from the transparent agar and transfer them to other media. Agar No. 5 through 8 were more solid and, therefore, had better physical properties at the 39° C. incubation temperature than Agars No. 1 through 4.

It was desirable to have faster colony growth than was achieved with agars 1 through 8. Since these media produced 0.2 to 0.5 mm. colonies in 48 hours and 2 mm. colonies in 4 weeks, it was hoped that an isolation medium could be found to produce large colonies in a short incubation time. Consequently, two new media were prepared containing mineral solution M. They are described below.

Mineral solution M

Calcium acetate		0.01	g.
Ammonium sulfite		0.02	g.
Magnesium chloride hexahydrate		0.01	g.
Monobasic potassium phosphate		0.02	g.
Dibasic sodium phosphate heptahydrate		0.05	g.
Urea		0.1	g.
Manganous chloride tetrahydrate		0.00025	g.
Sodium tetraborate		0.0005	g.
Cobaltous chloride tetrahydrate		0.0001	g.
Ammonium molybdate		0.00005	g.
Sodium carbonate		0.1	g.
Distilled water	to	40	ml.

Agar No. 9. Yeast extract - CMC-70L

Bacto yeast extract		1	g.
CMC-70L		1	g.
Cysteine hydrochloride		0.05	g.
Sodium thioglycollate		0.05	g.
Mineral solution M		40	ml.
Agar		1.75	g.
Distilled water	to	100	ml.

Agar No. 10. Yeast extract - CMC-70L - Cellobiose

Agar No. 10 is the same as agar No. 9 with the addition of 0.2 g. of cellobiose.

Duplicate tubes of sterile agars 9 and 10 were inoculated with a 10^{-4}, 10^{-6}, 10^{-8} or 10^{-10} dilution of a culture of organism No. 32 (see Procedure). After inoculation, each tube was gassed with carbon dioxide, cooled as a film on the sides of the tube and incubated at 39° C. for 96 hours. There was good growth in all tubes. In the tubes inoculated with a 10^{-10} dilution the colonies were 0.5 mm. in diameter for agar No. 9 and slightly larger, 0.75 mm., in agar No. 10. This is an indication that the cellobiose helps initiate and promote growth better than CMC-70L alone.

Microscopic examination of the colonies showed all colonies to be similar. The colonies were white and fingers of growth were protruding in all directions. The colony appeared to be shaped like a double convex lens to the naked eye.

Excellent growth was obtained in every case by picking colonies from the agar and transferring one colony to each of duplicate tubes of media 20 and 21 described below.

Medium No. 20. Yeast extract - CMC-70L

Bacto yeast extract		1	g.
CMC-70L		1	g.
Cellobiose		0.2	g.
Glucose		0.2	g.
Mineral solution M		40	ml.
Sodium thioglycollate		0.05	g.
Cysteine hydrochloride		0.05	g.
Resazurin dye		0.0001	g.
Distilled water	to	100	ml.

Medium No. 21. Yeast extract - Trypticase - CMC-70L

Difco yeast extract		0.5	g.
Trypticase		0.5	g.
Phytone		0.5	g.
CMC-70L		1	g.
Cellobiose		0.2	g.
Glucose		0.2	g.
Mineral solution M		40	ml.
Sodium thioglycollate		0.05	g.
Cysteine hydrochloride		0.05	g.
Distilled water	to	100	ml.

2. Nutritional characteristics

It was desired to find if organism No. 32 would grow on a medium composed of the amino acids, vitamins, minerals, and other metabolites which were readily available in this laboratory. Consequently, culture No. 32 was used to inoculate several media containing various dilutions of the basal medium described below.

Basal medium No. 1

DL-Alanine	42.5	mg.
L-Arginine hydrochloride	42.5	mg.
DL-Aspartic acid	42.5	mg.
Glycine	42.5	mg.
L-Histidine hydrochloride	42.5	mg.
DL-Isoleucine	42.5	mg.
L-Leucine	21.3	mg.
L-Lysine hydrochloride	21.3	mg.

Basal medium No. 1 (continued)

DL-Methionine		42.5	mg.
DL-Phenylalanine		10.0	mg.
L-Proline		42.5	mg.
DL-Serine		5.0	mg.
DL-Threonine		5.0	mg.
DL-Valine		42.5	mg.
L-Asparagine		15.0	mg.
L-Glutamine		20.0	mg.
L-Tyrosine		40.0	mg.
L-Glutamic acid		121.3	mg.
L-Tryptophan		5.0	mg.
Adenine sulfate		1.0	mg.
Guanine hydrochloride		1.0	mg.
Xanthine		1.5	mg.
Uracil		1.0	mg.
Magnesium sulfate heptahydrate		0.2	g.
Ferrous sulfate heptahydrate		0.01	g.
Manganous sulfate		0.0068	g.
Monobasic potassium phosphate		0.4	g.
Dibasic potassium phosphate trihydrate		0.524	g.
Pyridoxine		0.2	mg.
Pyridoxal		0.2	mg.
Nicotinic acid		0.2	mg.
Riboflavin		0.2	mg.
Calcium pantothenate		0.2	mg.
Thiamine		0.2	mg.
Biotin		0.001	mg.
Folic acid		0.011	mg.
Para-amino benzoic acid		0.008	mg.
L-Cystine hydrochloride		0.2	g.
Sodium acetate		1.2	g.
Sodium citrate		1.2	g.
Tween 80		0.2	g.
Glucose		4.0	g.
Distilled water	to	100.0	ml.

pH adjusted from 5 to 7 with dilute ammonium hydroxide

Several different dilutions were made of the basal medium. Duplicate tubes of each dilution were inoculated and duplicates left uninoculated. The ratio at which these dilutions were made is described below.

Medium No. 22. Chemically defined

Basal medium No. 1		50	ml.
Distilled water	to	100	ml.

Medium No. 23. Chemically defined

Basal medium No. 1		30	ml.
CMC-70L		1	g.
Distilled water	to	100	ml.

Medium No. 24. Chemically defined

Basal medium No. 1		20	ml.
CMC-70L		1	g.
Distilled water	to	100	ml.

Medium No. 25. Chemically defined

Basal medium No. 1		10	ml.
CMC-70L		1	g.
Distilled water	to	100	ml.

Medium No. 26. Chemically defined

CMC-70L		1	g.
Distilled water	to	100	ml.

No growth was observed in any of the dilutions of the basal medium (Nos. 22 to 26). This is an indication that the medium must lack a factor essential to the growth of organism No. 32 or that some material in the basal medium is toxic to the organism.

Several nitrogen compounds were tested to see if organism No. 32 would use them as a source of nitrogen. Equal nitrogen equivalents of the compounds were used. Duplicate tubes of each concentration of each compound were inoculated and duplicates were left uninoculated. It was hoped that the uninoculated tubes would provide comparable blanks which

could be used for the turbidimetric determination of growth by means of a photoelectric colorimeter. Since yeast extract was required in the medium in order to provide the unknown growth factor, it was hoped that these nitrogen compounds would produce growth above and beyond that produced by a minimal yeast medium. Unfortunately, it was found that the organism bleached the coloring material from the test medium. This made it impossible to check the turbidity on a photoelectric colorimeter because the absorption band changed with the amount of growth. It was not determined whether the discoloration was proportional to the amount of growth. The medium and compounds used are given below (see Table XIX).

Medium No. 27. Test medium

Bacto yeast extract		1	g.
CMC-70L		1	g.
Solution C		40	ml.
Nitrogen compound (see Table XIX)		-	
Distilled water	to	100	ml.

3. Growth factor characteristics

It is highly probable that organism No. 32 is very fastidious and requires many complex nutrients in its medium. That this is true, is borne out by the fact that the 42 ingredients in Basal Solution No. 1 did not support growth in any concentration tried in this investigation. The amount of each of these complex nutrients required for optimum growth by organism No. 32 is probably fixed between rather narrow limits. Since it was impossible to determine the completeness of Basal Solution No. 1, it was concluded that the best way to check for a

Table XIX

Utilization of nitrogenous compounds

Name	Concentration g. per 100 ml. of Medium[a]	Utilizability by organism No. 32[b]
Urea	0.300	S
Urea	0.150	S
Ammonium nitrate	0.40	S
Ammonium nitrate	0.20	S
Ammonium formate	0.63	S
Ammonium formate	0.315	S
Hexamethylenetetramine	0.35	I
Hexamethylenetetramine	0.125	I
Sodium nitrate	0.85	S
Sodium nitrate	0.425	S
Phytone	1.0	P
Phytone	0.5	P
Blank (no nitrogen besides yeast)		Standard

[a]The pure compounds were used on the basis of equal nitrogen content.

[b]The key to the utilization is as follows: S, same growth as yeast standard; I, inhibits growth; P, promotes growth above that of the standard.

possible unknown growth factor for organism No. 32 would be to measure the growth stimulation produced when the factor was added to a medium known to support minimal growth.

Consequently, a test was run with a series of yeast extract concentrations ranging from none to 5 per cent to determine the minimal growth concentration. The basic medium No. 28 described below was used as a test medium. (See Table XX.)

Medium No. 28

CMC-70L		1	g.
Mineral solution A		20	ml.
Solution C		20	ml.
Bacto yeast extract		varied	
Resazurin		0.0001	g.
Distilled water	to	100	ml.

Medium No. 28 was not suitable for photoelectric colorimeter comparison of turbidity. The inoculated tubes were bleached compared to the uninoculated blanks at each concentration. This changed the absorption spectrum. There was an increase in the light absorption with increase in yeast concentration. However, this had no connection with the growth of the organism.

One gram of Bacto yeast extract was ashed in a crucible at full Bunsen burner flame. This ash was dissolved in distilled water and the solution diluted up to 20 ml. This mineral solution was tested for the presence of the growth factor by using it to replace yeast extract in Medium No. 28, a medium in which organism No. 32 grew well. The Bacto yeast extract ash failed to support any growth of organism No. 32 when replacing yeast extract in the medium.

Table XX

Growth of organism No. 32 on yeast extract concentration series

Per cent yeast extract in Media	Growth
0	0
0.5	1+
1.0	1+
1.5	2+
2.0	3+
2.5	3+
3.0	4+
3.5	4+
4.0	3+
4.5	2+
5.0	2+

By ashing the yeast extract, all of the organic nutrients were destroyed. Yeast extract ash did not support growth of organism No. 32. This means that one or more of the organic factors were destroyed. The best way to check for a possible unknown growth factor would be to measure stimulation in growth of organism No. 32 in a medium known to support minimal growth. This was not done in this investigation, pending the development of a medium, not discolored by the bacteria, which could be used for turbidimetric assay.

Dialysis of yeast extract and dry torula yeast was performed to determine whether the nutrients required by organism No. 32 could have molecules small enough to pass through a dialysis bag. A 2-g. sample of Bacto yeast extract was dissolved in distilled water and diluted to 10 ml. This was tied in a sausage casing, dialysis bag, suspended in 15 ml. of distilled water and dialyzed for 24 hours in the refrigerator. In addition to this, a 3.5-g. sample of dry torula yeast was suspended in 20 ml. of distilled water and dialyzed against 20 ml. of distilled water for 24 hours in the refrigerator.

The dialyzate and the dialyzed material from both the yeast extract and torula suspension were diluted to 20 ml. When suspended in medium No. 28 at volume concentrations of 10 and 20 per cent, growth was obtained in every concentration with organism No. 32. This indicates that the nutrients required by organism No. 32 are dialyzable.

The torula extract described in agar medium No. 4 had the protein removed by precipitation with two volumes of methanol. When the

alcohol was removed, it supported growth of organism No. 32 in agar medium No. 4. It also supported growth of organism No. 32 in medium No. 29. Medium No. 29 was a broth identical in composition to agar No. 4 with the agar and yeast extract removed. It was used to test various concentrations of the yeast extract.

Medium No. 29. CMC-70L - Test broth

CMC-70L		1	g.
Cellobiose		0.2	g.
Mineral solution K		40	ml.
Yeast factor (to be added)			
Distilled water	to	100	ml.

Two attempts were made to remove the growth factor(s) from torula yeast extract by the use of ion exchange resins. In the first attempt, 50 ml. of deproteinized torula extract was placed in a beaker with Nalcite HCR cation exchange resin. The resin had been freshly regenerated with 4 per cent sulfuric acid and back washed with distilled water to pH 5.0. The torula extract was agitated with this resin until the pH was lowered to 4.3. The extract was then decanted into a beaker containing freshly regenerated amberlite IRA-410 anion exchange resin which had been back washed with distilled water to pH 6.

The torula extract was agitated with the amberlite IRA-410 until the pH had risen to 8.0. It was then decanted back to the cation resin. This decanting was repeated until the torula extract failed to rise above pH 7 with the anion exchange resin. Samples of the torula extract were saved from the first, second, third and last decanting. The pH was adjusted to 7 with acetic acid or ammonium hydroxide and

then tested to see if the unknown factor had been removed by the resin treatment.

A 500-ml. sample of the deproteinized torula extract was passed through both a cation and an anion exchange resin column. The resins were the same as those used in the experiment described above. Both were freshly regenerated, and the resin column in both cases was 3 x 32 cm.

Medium No. 29 was used as the test medium to determine whether the ion exchange resins had removed the growth factor from the torula extract. In every case, the treated extract gave about the same growth response as the untreated torula extract. This indicates that the factor(s) was not removed by the ion exchange treatment used in this investigation. The ion exchange resins did not remove the color from the yeast extract. Consequently, it was no better than the original torula extract for preparing a colorless medium for turbidimetric assay.

An experiment was run to find the relative solubility of the unidentified factor(s) in water and alcohol. In this experiment, 10-g. samples of dry torula yeast were extracted by agitating 40 ml. of solvent with the yeast in a 50-ml. centrifuge tube. The mixture was then centrifuged and the extract decanted. An equal amount of solvent was added to the residue, and the extraction process was repeated.

In each extraction where the methanol concentration was below 50 per cent, each fraction was deproteinized by boiling with 2 volumes of methanol, the protein was removed by centrifugation and the methanol was boiled out. The extract was then made up to 40 ml. with distilled water.

Four extractions were made of the yeast samples extracted with hot water and cold water. Only one extraction was made with the alcohol solvents. Each extract was tested for its ability to support the growth of organism No. 32. Test medium No. 29 was used to supply the carbohydrate and minerals. It was inoculated as described in the Procedure section. The results are given in Table XXI. Here it is shown that the factor is about as soluble in methanol as in water, in methanol concentrations up to 50 per cent. There is a slight solubility of the factor(s) even in 100 per cent methanol.

Three water solutions of Bacto yeast extract were steam distilled in order to determine whether the growth factor(s) could be distilled this way. Two 2-g. samples of Bacto yeast extract were dissolved in approximately 20 ml. of distilled water. These duplicate samples were at the natural pH of 6.0. Another 2-g. sample of Bacto yeast extract was dissolved in distilled water and adjusted to pH 1 with sulfuric acid. These samples were steam distilled, and 40 ml. of distillate were collected from each. The residual liquor in the distilling flask was also diluted to 40 ml. Medium No. 29 was used as the test medium. The steam distillate and distilled residual liquor were used at

Table XXI

Solubility of cellulolytic factor(s) in water and methanol

Solvent	Relative Growth[a]
Hot Water (100° C.)	
1st extract	3+
2nd extract	3+
3rd extract	2+
4th extract	1+
Cold Water (25° C.)	
1st extract	2+
2nd extract	2+
3rd extract	1+
4th extract	1+
Methanol	
25 per cent	2+
50 per cent	2+
75 per cent	1+
100 per cent	1+
Water blank replacing extract in medium No. 29	N

[a]The symbols used denote relative growth: 1+ represents the least and 3+, the most growth. N denotes no growth.

concentrations corresponding to 1 and 0.5 per cent by weight of the yeast extract from which they were prepared. In every case the residual liquor from the steam distillation supported approximately the same amount of growth as standards prepared from Bacto yeast extract that was not distilled. None of the steam distillates supported any growth. This indicates that the cellulolytic factor(s) are not removed from water solution, at pH 1 or pH 6, by steam distillation. The coloring matter was not removed from the extract by steam distillation either.

An experiment was performed to see whether the growth factor(s) for organism No. 32 could be extracted from yeast extract with ether at pH 2. It was hoped that at pH 2 any carboxylic acid salts would form carboxylic acids and be ether soluble. Consequently, 5 g. of Bacto yeast extract was dissolved and made up to 20 ml. volume with distilled water. The solution was adjusted to pH 2 with hydrochloric acid. This solution was then extracted with five 20-ml. portions of ether by shaking each extraction about 4 minutes in a separatory funnel.

The ether fractions were combined and evaporated in a hood. The residue from the ether fraction and the ether extracted residue were each made up to 20 ml. volume with distilled water. The ability of these two fractions to sustain the growth of organism No. 32 was determined using test medium No. 29. The ether extract did not sustain growth. The ether insoluble fraction supported growth comparable to unextracted yeast extract. This indicates that none of the essential growth factors is ether soluble.

According to Quinn (195) and Ruf (215) the cellulolytic growth factor that they were investigating could be adsorbed on Norite A at pH 3 and eluted at pH 7. In this investigation similar results were obtained. A 25-ml. sample of torula yeast extract of pH 5, deproteinized with methanol and the methanol evaporated (see description of agar No. 4) was treated with 1 g. of Norite A in a beaker. The carbon was removed by filtering the material through a Seitz filter. The carbon was washed with water at pH 5. A 1/15 M phosphate buffer of pH 7 was used to elute the Norite A.

The carbon adsorbed yeast extract and the colorless buffer elutate were tested for their ability to sustain the growth of organism No. 32. Both supported growth equally well (2+) but not as well as untreated torula extract (3+). This should signify that the growth factor(s) can be adsorbed on Norite A at an acid pH and eluted at pH 7.

The buffered carbon eluate was colorless. Test medium No. 29 was also colorless. Further work with this combination should provide a medium which could be used satisfactorily for turbidimetric analysis of the nutritional requirements of rumen organism No. 32. The results of such experimentation may be applicable to the nutritional requirements of cellulolytic rumen organisms <u>in vivo.</u>

VI. GENERAL DISCUSSION AND CONCLUSIONS

A. Dry Bland Yeast Protein Concentrates

There is much evidence (50) that yeasts contain many of the valuable food constituents which are essential in the diets of men and animals. However, the use of yeast as human food, especially in quantities sufficient to supply a significant amount of the daily protein, has been limited by its unpleasant taste (31). Since high-quality protein is the limiting factor in the world's food production, yeast protein, if highly acceptable, would be a valuable addition to the food resources of the world.

This investigation was conducted primarily on the basis that protein molecules are very large and should be relatively tasteless, and that any unpleasant taste in yeast should be due to the small molecules associated with the protein. The problem then became one of finding a method of separating the low molecular weight, taste-producing substances from the protein. One of the first tasks was to find a way to break the yeast cell wall and, thus, make the cell constituents available for processing.

The investigation was begun in the hope that it would be possible to remove all of the protein from the yeast cells by an exploding process similar to that used by Anderson (7) and Mason (160) for exploding cereal grains and wood. The principle is to heat a slurry of yeast cells in a closed system to a temperature above the boiling point of the intra-cellular liquids at atmospheric pressure. The system is

then opened to the atmosphere in such a manner as to allow the external cell pressure to be released more rapidly than the internal cell pressure can be dissipated through the intact cell. The resulting pressure differential should rupture the cell, provided that the internal temperature had been high enough.

Three devices were constructed to determine whether the conditions of time, temperature and pressure employed would be suitable for adapting the exploding principle to the preparation of bland protein concentrates from yeast. It was found that the exploding effect was negligible under the experimental conditions used, and that the use of higher temperatures than those employed would result in a scorched protein product.

It was demonstrated that up to 79.9 per cent of the total nitrogen could be released from the yeast by exploding it from a batch autoclave at 160 p.s.i. steam pressure. It was also demonstrated that a regular increase in exploding temperature resulted in a proportional increase in the amount of nitrogen released from the cells. However, by using a continuous yeast exploding device it was proved that the above nitrogen release was due to the heating effect rather than the exploding effect at temperatures below 140° C. Moreover, in almost every instance in this investigation, except when less than 0.26 minutes' heating time was used, the product was scorched when the temperature was allowed to rise above 160° C.

When yeast was exploded using a continuous, steam-heated heat exchanger followed by release from an orifice, it was found that from 16.5 to 60.5 per cent of the nitrogen could be released as the exploding temperature was increased from 100° to 160° C. However, the greatest amount of protein nitrogen released, as determined by alcohol precipitation, was 9.83 per cent. This was positive evidence that it would be impractical to attempt to take advantage of the exploding effect in producing bland proteins from yeast.

The theory for producing a bland yeast protein was to remove the low molecular weight substances from the protein. Exploding the cells at high temperature degraded the protein molecules. This tended to increase the amount of taste-producing small molecules and at the same time lowered the yield of tasteless protein below 10 per cent of the available nitrogen. This low yield precluded further investigation of the industrial potentialities of complete removal of the protein from yeast by exploding. It might have been possible to remove the protein from cells which had only been slightly altered by the exploding by using salt or alkaline solutions but that would have been a costly step--a step which it was desired to eliminate.

The emphasis of the investigation was diverted to determining whether it would be possible to leave all of the protein in the cell and change the permeability enough to allow the small molecules to be extracted. Heat seemed to be the most rapid and least expensive way of altering the cell wall permeability. Heating the yeast cells had the advantage of not introducing extraneous chemicals which would require

neutralization or removal from the final product. Heating could also be done rapidly in a continuous heat exchanger. Heating, just like autolysis, hydrolysis or other methods of breaking yeast cells, involves a balance between the destruction of the cell constituents and the achievement of the desired cell wall permeability.

Preliminary experiments showed that ethanol was a more satisfactory solvent than water, vegetable oils or fat solvents for extracting the taste-producing small molecules from heated yeast. Consequently, alcohol or a mixture of alcohol and hexane was used to test the applicability of heating as a means of changing the cell wall permeability of both bakers' and torula yeast.

It was found that a very bland, yeast protein concentrate containing between 50 and 60 per cent protein, could be produced by heating a yeast slurry to 120° C. in a heat exchanger and extracting the taste-producing substances with 95 per cent ethanol. The most satisfactory method used was to heat the yeast slurry continuously in a steam-heated heat exchanger. A slurry containing 13.5 per cent of yeast (weight of dry solids) was handled very easily, and slurries of higher concentrations could have been handled. Heating the yeast slurry from 0.97 to 2.22 minutes at 120° C. altered the cell wall permeability sufficiently to allow the alcohol to extract the taste-producing molecules. However, heating the yeast slurry to 100° C. for 0.72 to 1.85 minutes did not allow the extraction of as much of the taste as had heating at 120° C.

Based upon the success which was achieved in the alcohol extraction of the taste from yeast in this investigation, a flow sheet is proposed in Figure 8 for the continuous treatment of a yeast slurry for the production of a dry, bland protein concentrate. This is a relatively simple process which employs equipment commonly used in the yeast and alcohol industries. Other schemes may prove to be more practical. This scheme was suggested because the material is handled in fluid form in all steps except the packaging step.

The reason for producing, protecting and delivering a dry, bland yeast protein is that protein is needed for human consumption. Yeast protein must be acceptable by humans before they will consume it. It must be consumed before it is of any nutritional benefit. One of the reasons for removing the taste from yeast is to make it more acceptable. There is not a great deal of necessity for removing the taste from yeasts for animal feeding except in special instances, i.e., the preparation of purified proteins for experimental purposes.

In order for dry, bland yeast proteins to be accepted and made of the greatest ultimate value for human nutrition, there are several aspects which deserve consideration and research. The following outline is suggested as a logical way to break the problem down into steps which can serve as a guide for literature survey and research. This outline is not intended to be complete, inflexible or dogmatic.

Dry, bland yeast protein problems can be broken down into four categories, namely, problems of production, protection, distribution and

utilization. Each should be investigated by considering every aspect of safety, quality, quantity, unit cost and morale or acceptance by the individual humans involved. Usually, the satisfactory solution of the problems of safety, quality, quantity and unit cost makes it much easier to solve the problems of acceptance by the individuals involved. This also implies that due concern for the individual should be exercised in seeking the answers to these problems. This, undoubtedly, places the highest priority on those problems involving the utilization of yeast proteins by the individual.

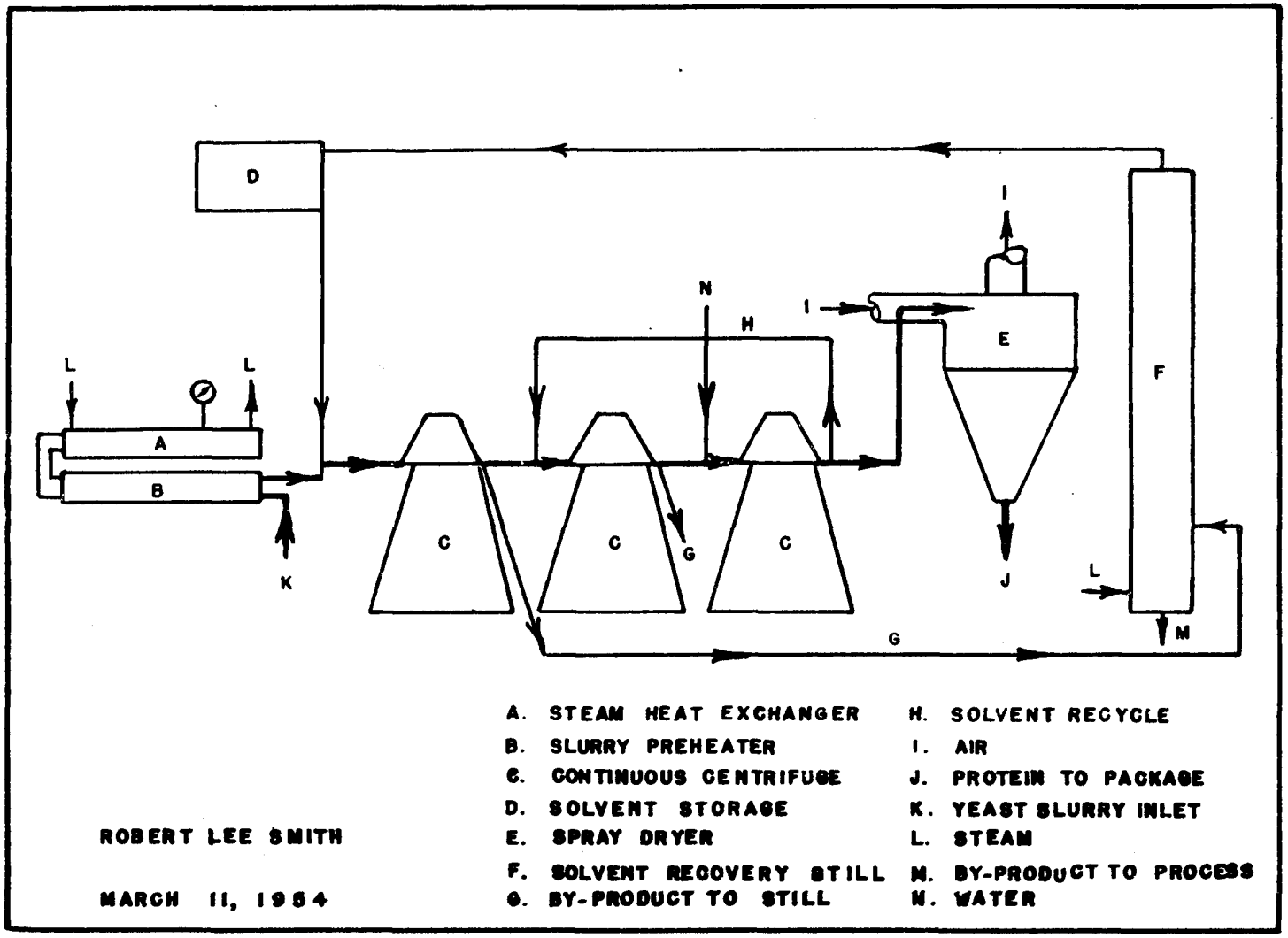
1. Production

Figure 8 is a proposed flow sheet for altering yeast cells and extracting the taste-producing, low molecular weight substances. This flow sheet is based on the experience gained in this investigation. Other procedures can be devised and the virtues and modifications of each should be compared. Primarily, production problems will involve the practical application of engineering principles to the theoretical principles of bacteriology and chemistry which have already been worked out.

a. Safety

(1). Properly designed equipment and due precautions are needed for the safe handling of the inflammable alcohol or other solvents used.

Figure 8. Flow sheet of a proposed continuous process for altering the cell wall permeability and extracting the taste from yeast to produce dry, bland yeast protein concentrates



FLOW SHEET FOR DETASTING YEAST PROTEIN

(2). The equipment should be constructed of materials which will not introduce toxic matter into the product.

(3). Production of the yeast raw material and every phase of production should be conducted with due regard for elimination of undesirable contamination from micro-organisms and extraneous material.

(4). Raw materials for yeast manufacture should be analyzed to determine if toxic materials are present.

(5). Adequate accessory equipment should be available for protection of personnel, i.e., fire protection, fume masks, explosion-proof lights and motors, ventilators, etc.

b. Quality

The quality of proteins for human consumption is based on many factors. Proteins have such large molecules that they are relatively tasteless and odorless. However, conventional protein commodities are an association of chemical entities of such a nature that these commodities are currently being purchased on the basis of taste, texture, smell, sight, appeal and other intangible qualities of eating enjoyment. Biological nutritive quality of protein is only vaguely considered at the retail level.

(1). The product under consideration is a dry, bland protein from yeast, and the degree of blandness required will depend upon the way in which it is used. It should be possible to "tailor make" yeast protein concentrates to fill the need. This will be dependent upon the unit cost required to attain the various degrees of blandness. The quality specifications for the different protein concentrates will be dictated by the nutritional practices of the consumer, i.e., spicy foods can mask yeast protein concentrates which have a stronger taste than can bland foods. As soon as quality specifications have been established for a particular protein concentrate, methods must be devised and applied to maintain production quality control. This need for quality control exists not only for the yeast detasting process but also for the process of enriching yeast protein concentrates with synthetic amino acids or blending them with other proteins to improve their biological nutritive quality.

(2). The yeast heating phase in the production of the protein concentrate will need to be carefully investigated and controlled. Heat has a greater effect on protein quality than have any of the other unit processes involved.

(3). Care must be exercised in handling the live yeast to avoid autolysis and contamination. Both may tend to lower the quality of the protein product.

c. Quantity

(1). It is important to survey the raw materials available for conversion into yeast. The amount of yeast available for processing to dry, bland protein will depend upon the amount of indigenous agricultural, industrial and forest materials which can be utilized as the carbohydrate energy source for yeast growth.

(2). The processing equipment for converting yeast to protein concentrates should be designed for optimum capacity for the investment involved and should be designed for expansion to fit the consumer's demands.

(3). The equipment should have the degree of flexibility needed to supply the right quantity of protein concentrates of various quality specifications as demanded.

d. Unit cost

After due consideration has been given to the aspects of safety, quality and quantity, it is important that the cost be investigated. This means that the production process should be constantly improved and made more efficient in order to lower the cost of the product.

(1). Materials handling must be considered. When possible, it will be desirable to use yeast directly from the fermentor, after a preliminary concentration step, rather than use a refrigerated storage tank.

(2). Equipment construction materials must be picked with economy in mind, as well as safety and quality.

(3). Heat and power conservation are important. Proper choice of heat exchangers and electrical equipment will help insure this.

2. Protection and distribution

The problems of protecting and distributing the bland yeast protein, once it has been produced, may also be broken down into the various aspects of safety, quality, quantity and unit cost. Many of these problems have already been encountered and solved by the dry milk distributors. Since most of these problems will not be unique to the yeast protein concentrate industry, they will not be discussed here. Undoubtedly, work will need to be done to determine whether any quality deterioration occurs during storage.

3. Utilization

Dry, bland yeast proteins will probably be most useful as protein supplements to enrich various high-carbohydrate, fat and protein foods. Many of the problems of utilization will involve the nutritional

aspects of using protein concentrates to adequately enrich human diets. The acceptability of these foods by the consuming public will be dependent upon how well the scientists, engineers and economists can answer the questions of safety, quality, quantity and unit cost. It is, therefore, imperative that adequate and objective standards of safety, quality and quantity be established for proteins from various sources, not only to guide and improve research on protein concentrates but to provide a vocabulary for adequately representing all proteins in commerce.

a. Safety

(1). Alcohol is considered to be a safe solvent to use with food products. However, animal feeding tests should be performed on the bland yeast protein to determine if any toxicity is present due to unknown reactions which might occur in processing.

(2). It is known that yeasts are deficient in Schwarz factor No. 3 (226). Extraction of yeast with ethanol to produce bland proteins may make the protein concentrate deficient in several factors. This aspect should be investigated by animal studies to determine safe ways to blend the protein with other food constituents and food commodities.

(3). The yeast used as raw material and the protein product should be investigated for disease-producing contaminants in a manner approved by the food industry.

b. Quality

(1). The amino acid content of the protein should be determined.

(2). The digestibility of the protein and the availability of the various amino acids should be determined.

(3). The methods of improving biological nutritive quality by synthetic amino acid enrichment and by blending with other proteins, i.e., fish meal, should be determined.

(4). The protein concentrate should be analyzed for all of the other food constituents, namely, carbohydrates, fats, vitamins and minerals. Alcohol extraction of yeast would be expected to remove any free B-vitamins but those which were conjugated with the protein might remain.

(5). The taste quality is an important consideration. It would be very interesting to know which compounds are responsible for the strong yeast taste. Carter (34) states that the free amino acids in yeast account for as much as 7 per cent of the total nitrogen present. One has only to taste the various amino acids to become convinced that the free amino acids, especially tryptophan, lysine, threonine and histidine, could account for some of the taste in yeast.

(6). Smell and taste are dependent upon the small molecules associated with the protein. Research, therefore, needs

to be done to determine a variety of ways of blending the dry, bland yeast protein into other foods and seasoning them to achieve the best combinations for good taste, smell, texture and sight appeal.

c. Quantity

(1). It is important to know and be able to communicate to the consumer the quantity of available protein, as well as the quantity of each amino acid in the yeast protein concentrate.

(2). Recipes are needed which will indicate the quantity of yeast protein needed to blend with other proteins to result in a food's meeting desired protein quality specifications, i.e., a protein with a biological nutritive quality equivalent to egg protein.

(3). Recipes are needed to make high calorie foods such as potatoes, cereals, pastries and the like, rich in protein. Judicious use of bland protein concentrates may make it possible to change fattening foods to reducing foods by decreasing the number of calories per gram of protein to that recommended by doctors for reducing diets.

d. Unit cost

It is important that those involved in the production, protection, delivery and utilization of the yeast protein

concentrate should know the unit cost of the constituent protein, as well as the cost of the commodity. This knowledge will make it possible to compare the costs of producing, protecting and delivering yeast constituent proteins with proteins from other food commodities.

Yeast proteins are not of animal nutritive quality and, therefore, should not be compared with the animal proteins shown in the price report illustrated in Figure 9 until suitable protein biological nutritive quality standards have been established in commerce. This protein price report is distributed by the Protein Economics and Research Council, Inc. (194). It should be possible to blend yeast protein concentrates with amino acids and proteins from other sources to produce a protein mixture with a high biological quality whose price could be sensibly compared with the protein prices in Figure 9. Work is needed to produce high quality-low cost protein blends.

B. Unknown Factor(s) Which Stimulates Cellulose Digestion by Rumen Organisms

The by-products from the process described in Figure 8 for preparing the bland protein concentrate from yeast will be found in the water-ethanol extract. This extract will, undoubtedly, contain free amino acids and small peptides, vitamins, any alcohol soluble fats and carbohydrates, as well as some of the chemical constituents which have been listed in Table VI. These by-products are valuable. Some are

Figure 9. The protein price report--
a way to compare the cost of producing,
protecting and distributing animal proteins
from different sources

RETAIL PROTEIN PRICE REPORT FOR U. S. A.
February 15, 1954

Food	Retail Price[1]	% Protein[2]	Price per lb. of Protein[3] Paid by Consumer[4]
Beef—			
Chuck Roast	$0.54 lb.	16.2	$3.07
Hamburger	0.42 lb.	16.1	2.08
Round Steak	0.86 lb.	17.6	4.72
Cheese—			
Cottage	0.24 12 oz. box	19.5	1.63
Cured Cheddar	0.68 lb.	25.1	2.32
Chicken—			
Frier, ready to cook	0.60 lb.	15.2	3.83
Eggs, Fresh	0.68 doz.	11.4	3.90
Fish—			
Haddock, Frozen Fillet	0.48 lb.	18.2	2.64
Salmon, Canned Pink	0.53 lb.	20.5	2.49
Lamb, Leg	0.75 lb.	15.0	4.71
Milk—			
Evaporated	0.15 14½ oz. can	7.0	1.86
Fresh, Whole	0.22 qt.	3.5	2.48
Non-fat, Dry	0.40 lb.	35.6	0.97
Pork—			
Chops	0.81 lb.	13.3	5.62
Ham, Whole	0.65 lb.	14.7	3.85

[1] Estimated retail prices reasonably representative of current U. S. prices on basis of spot checks and price trends. Must be adjusted to meet local conditions.

[2] Percent protein, Composition of Foods, U. S. Dept. of Agriculture, Agriculture Handbook No. 8. Foods may vary appreciably from these values.

[3] *In estimating the price per lb. of protein in the above foods, all carbohydrates are arbitrarily calculated at 10c per lb. and fats at 30c per lb. The dollar value of vitamins, minerals and water is considered negligible on a pure chemical market basis. The balance is charged against protein.*

[4] The proteins considered in this report are all of animal protein nutritive quality.

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valuable nutritionally; others are valuable chemical or biological raw materials. For the economy of the over-all process, these by-products should be utilized to the best advantage.

In the course of this investigation, it was determined that one of the by-products in the water-alcohol extract of both torula and bakers' yeast was a growth factor(s) which promotes cellulose digestion by rumen organisms. This factor(s) may prove valuable in ruminant nutrition.

It is a well-known fact that the cellulosic feedstuffs which ruminants consume are degraded in the rumen by the microorganisms which abound there. Kitts (124) and Ruf (215) have given literature reviews and reported their work with the artificial rumen technique for using rumen organisms to degrade cellulosic materials in vitro. Ruf (215) and Quinn (195) have shown that there is a factor(s) in yeast and other feedstuffs which promotes cellulolytic digestion by rumen organisms. Kitts (124) has shown that the cellulose is degraded by an enzyme system which is closely associated with the bacterial cells and is not found as an exoenzyme. He (124) also pointed out that CMC-70L was used as a cellulose source by the rumen organisms.

In the course of this investigation several CMC-70L agar media were prepared which made it relatively easy to isolate organisms using CMC-70L as a sole source of carbohydrate. Since CMC-70L is a sodium salt of one of the less substituted carboxymethylcellulose derivatives, it is water soluble and forms a colorless, transparent solution with a

low viscosity in low concentrations. As a consequence, these media were transparent and contained no insoluble particles. Colonies were easy to see and transfer.

Several rumen organisms were isolated and one (organism No. 32) was used to develop anaerobic techniques and media for analyzing some of the characteristics of the yeast factor(s) which promote cellulose digestion. Organism No. 32 was isolated from the rumen of a Holstein cow. An electron microphotograph of organism No. 32 was shown by Kitts (124). Microscopic examination of cultures of organism No. 32 revealed motile, rod-shaped bacteria. They were Gram variable. Young cultures were Gram negative and older cultures tended to be Gram positive. Organism No. 32 actively digested cellulose, starch, dextrin, glucose, maltose and cellobiose. The colonies of organism No. 32 were white, biconvex lens shaped in CMC-70L agar medium. Microscopic examination revealed fingers of growth from the colony rather than a solid mass of growth. Organism No. 32 was a strict anaerobe and 48 hours' incubation time at 39° C. was required for good colony growth with the best medium developed. Even then the colonies were only 0.5 to 0.75 mm. in diameter.

Organism No. 32 did not grow on a chemically defined medium containing the amino acids, vitamins, purines, pyrimidines minerals, glucose and CMC-70L which were available in this laboratory and commonly used in bacterial media. Therefore, several attempts were made to determine some of the characteristics of the factor(s) needed to support growth of organism No. 32. These characteristics are as follows:

a. The factor(s) is found in Bacto yeast extract, torula and bakers' yeast.

b. The factor(s) is soluble in water and in alcohol-water solutions. It is even slightly soluble in 95 per cent ethanol. This makes it possible to deproteinize yeast extracts with ethanol without losing the factor.

c. Ashing Bacto yeast extract was found to destroy the factors needed for growing organism No. 32.

d. The factor(s) could be dialyzed.

e. The factor(s) could not be adsorbed on Nalcite HCR cation exchange or Amberlite IRA-410 anion exchange resins.

f. It was not possible to steam distill the factor(s) at pH 6 or pH 1.

g. The factor(s) was not soluble in ether.

h. It was possible to adsorb the factor(s) on Norite A at pH 5 and elute it at pH 7 with 1/15 M phosphate buffer.

i. Optimum growth of organism No. 32 was found when the medium contained between 3 and 3.5 per cent of Bacto yeast extract. The organism seemed to be inhibited by concentrations of extract greater or less than 3 to 3.5 per cent.

The isolation and study of anaerobic cellulolytic rumen organisms, such as organism No. 32, should help in understanding the cellulose

digestion processes which occur in ruminants. By knowing all of the growth factors required by the cellulolytic rumen organisms, it should be possible to supplement ruminant rations to obtain optimum feed efficiency.

The clear, transparent cellulose agars made with CMC-70L should make it relatively easy to isolate cellulolytic organisms. The investigation of the nutritional requirements may be facilitated by the development of quantitative biological assay procedures. The results of this investigation suggest two possibilities for doing this.

Organism No. 32 produced acid from the CMC-70L cellulose derivative. Phenol red did not appear to inhibit the organism in low concentrations. Brom thymol blue or some other indicator might prove to be more practical than phenol red. However, by using an indicator it should be possible to assay the factor(s) by titrating the acid formed when organisms similar to No. 32 are grown on media containing various concentrations of the growth factor(s).

The deproteinized extracts of bakers' and torula yeast and Bacto yeast extract were golden-brown in color. This color was bleached by the growth of organism No. 32, thus changing the light absorption spectrum. This made it impossible to use a photoelectric colorimeter to check growth by a turbidimetric assay, even though the CMC-70L medium was transparent. This color in the medium was not removed by steam distillation, treatment with ion exchange resins or ether extraction. However, the color was removed by adsorption on Norite A at pH 5. The

growth factor(s) was eluted as a colorless solution with 1/15 M phosphate buffer at pH 7. This colorless solution of the factor(s) should make it possible to use a turbidimetric assay for further identification of the factor(s). Evidence for this is that the colorless mineral medium (M) developed in this investigation and the colorless CMC-70L cellulose source combined with the colorless Norite A eluate of yeast extract provided sufficient growth factors to support the growth of organism No. 32.

VII. SUMMARY

1. Three exploding devices were constructed to determine whether yeast cells could be exploded in such a manner as to release all of the cell protein under conditions of time, temperature and pressure which would be suitable for the commercial preparation of protein concentrates from yeast.

2. It was demonstrated that up to 79.9 per cent of the total nitrogen could be released from yeast by exploding it from a quick-opening autoclave at 160 p.s.i. steam pressure. It was also demonstrated with the quick-opening autoclave and with each of the continuous exploders (devices which heated the yeast slurry continuously and then released it through an orifice) that a regular increase in exploding temperature resulted in a proportional increase in the amount of nitrogen released from the cells. It was found that 16.5 to 60.5 per cent of the nitrogen could be released from the cells as the temperature was increased from 100° to 160° C. However, the amount of protein nitrogen released, as determined by alcohol precipitation and Kjeldahl analysis, was between 3.76 and 9.83 per cent.

3. A bakers' yeast slurry was forced through a steam-heated heat exchanger and then released through an orifice to the atmosphere. The amount of nitrogen released by this procedure reflected the nitrogen released by the heating effect plus the exploding effect. This amount was compared with the nitrogen released by the heating effect alone. The latter was ascertained by inserting a condenser between the heat

exchanger and the orifice. The condenser cooled the yeast slurry below 100° C. and eliminated any pressure differential across the cell wall due to vaporization of the intracellular fluids.

4. It was found that the nitrogen released from the cells was due to the heating effect and not the exploding effect from 100° to 160° C. Moreover, in almost every instance in this investigation, except when less than 0.26 minutes' heating time was used, the product was scorched when the temperature was allowed to rise above 160° C. On the basis of these results it was concluded that it would be impractical to attempt to take advantage of the exploding effect in producing bland proteins from yeast.

5. Experiments with both torula yeast and bakers' pressed yeast indicated that ethanol was a better solvent than water, vegetable oil, or fat solvents for removing the taste from yeast protein.

6. It was not possible to remove the scorched taste from drum-dried torula yeast. However, slurries of bakers' yeast or torula yeast when heated to 120° to 126° C. for less than 3 minutes had the permeability of the cell wall altered enough to allow the low molecular weight, taste-producing substances to be extracted with ethanol. For example, a torula yeast slurry containing 13.5 per cent solids was heated for 1.31 minutes at 126° C. The solids were recovered by centrifugation and extracted with 95 per cent ethanol. The product, when air dried, was creamy-white, very bland and contained 59 per cent protein. This represented a recovery of 76.5 per cent of the total nitrogen.

7. One of the by-products from the production of bland yeast protein concentrates is a water-alcohol soluble factor(s) which promotes cellulose digestion by rumen organisms. During the course of this investigation a rumen isolate was used to confirm the following characteristics for this factor(s).

a. It is found in torula and bakers' yeast and in Bacto yeast extract.

b. It is soluble in water and in mixtures of water and alcohol. It is slightly soluble in 95 per cent ethanol.

c. Ashing destroys the factor(s).

d. The factor(s) could be dialyzed.

e. It could not be adsorbed on Nalcite HCR cation exchange resin or Amberlite IRA-410 anion exchange resin.

f. It was not possible to steam distill the factor(s) at pH 1 or pH 6.

g. It was not soluble in ether.

h. It was possible to adsorb the factor(s) on Norite A at pH 5 and elute it at pH 7.

8. In order to facilitate investigation of the cellulolytic factor(s), several rumen organisms were isolated. Organism No. 32, a motile, strictly anaerobic, Gram variable, rod-shaped bacterium was

used as an assay organism. It actively fermented and produced acid from cellulose, CMC-70L, starch, dextrin, glucose and cellobiose.

9. A technique was developed for growing anaerobic inoculation cultures in centrifuge tubes. This procedure allowed the cultures to be easily centrifuged, washed and transferred to the test medium without becoming aerobic.

10. Several successful agar media were developed for isolating cellulose digesting organisms. The media used CMC-70L as a colorless, water soluble, cellulosic substrate. This resulted in a transparent cellulosic medium which made it easy to observe and transfer colonies.

11. Optimum growth of organism No. 32 was found when the medium contained between 3 and 3.5 per cent of Bacto yeast extract.

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