FACTORS AFFECTING THE ACTIVITY OF BAKER'S COMPRESSED AND ACTIVE DRY YEAST

A thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Biotechnology at Massey University.

STEPHEN ANGUS ESPIE

1980

ABSTRACT

Factors affecting the Activity of Baker's Compressed and Active Dry Yeast.

Parameters important in the production of Baker's Yeast were correlated with the product's final activity. Activity was a measure of the gas evolved in a fermenting dough, expressed as $mMCO_2$/hr/g yeast solids.

The drying of Compressed Yeast to Active Dry Yeast was optimised in terms of the humidity of the drying air. A tunnel tray drier was used to dry yeast to a 9% moisture content (dry weight). At 40°C. the optimum drying humidity was found to be 30-32% relative humidity. The leavening ability of yeast dried at 17% and 45% relative humidity decreased. A drying additive, 2% glyceryl monostearate, halved the drying time to 4 hours. Equations were developed to describe these observations as a function of relative humidity, drying time and additive concentration. The equilibrium relative humidity of stored dried yeast was found to be 32% at 20°C.

Fermentation parameters were correlated with the activity of Compressed Yeast using an experimental design. Growth temperatures varied from 28°C. to 37°C., initial pH from 4 to 6, glucose concentrations from 0.5% to 3%, nitrogen concentrations from 0.3% to 1.2% and dissolved oxygen varied as either agitated or standing cultures. Factors significantly affecting cell yield and yeast activity were growth temperature, dissolved oxygen and glucose concentrations. Maximal yeast activity occurred at 0.5% glucose concentration, 28°C. and non-agitated conditions. A model was developed to describe yeast activity as a function of these variables. The observed optimal conditions for cell yield were similar to those for yeast activity except for the dissolved oxygen level. Maximum yeast activity of Compressed Yeast occurred in non-agitated fermentations, compared with cell yield which required agitated conditions to achieve the greatest cell yield.

A rapid screening test for evaluating dried yeast was incorporated into the yeast activity analysis. This involved monitoring foam production during rehydration.

ACKNOWLEDGEMENTS

I wish to thank the following:-

Dr. G.J. Manderson for his encouragement and supervision throughout my Masterate. His advice and guidance has been most beneficial and was greatly appreciated.

Mr. I.W. Warrington of the Plant Physiology Division of the D.S.I.R. I am indebted for the facilities that were made available to me. The controlled environment rooms made possible the detailed studies on relative humidity and temperature described in this report.

Fermentation Industries, N.Z. Ltd. The supply of Compressed Yeast and molasses throughout this study was greatly appreciated.

Professor R.L. Earle for the provision of research facilities and the staff members of the Biotechnology Department, Massey University, for their help at various stages throughout my Masterate.

The trustees of the Brooker research award (1978) for their contribution towards the funding of this research.

N.Z. Forest Products Ltd., for the analysis of media sample for Total Carbon.

Dr. J.D. Brooks for helpful comments and proof-reading this thesis.

Mrs. M. Donald for typing this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND NOMENCLATURE

Ac	Yeast activity ($mMCO_2$/hr/g yeast solid)
ADY	Active Dry Yeast
AMIF-72	American Meat Institute Foundation (antioxidant no. 72)
Aw	Water activity
$\frac{dW}{d\theta}$	Rate of drying kg/S/m^2 (kg dry product)
EM	Emulsifier concentration $\frac{\text{kg emulsifier}}{\text{kg yeast slurry}}$
ERH	Equilibrium Relative Humidity
hc	Surface Heat Transfer Coefficient W/m^2 ^oC.
h_{fg}	Latent heat of evaporation J/kg
Hr%	Relative Humidity (percentage)
P	Pressure kPa
r.p.m.	Revolutions per minute
S.G.	Specific gravity
S.T.P.	Standard Temperature and Pressure
t	Temperature ^oC.
td	Dry Bulb temperature ^oC.
ts	Surface temperature ^oC.
tw	Wet Bulb temperature ^oC.
T.S.	Total solids
V	Gas volume mls.
V.m.	Monolayer capacity gH_2O/100g dry substance
v	Velocity m/sec.
W	Moisture content $\frac{\text{Kg water}}{\text{Kg dry product}}$
W_F	Final moisture content
Ys	Yield coefficient
β	Coefficient in an experimental design model
θ	Time of drying (hrs.)
θ_r	Relative time of drying
Δ G'	Change in free energy kJ
NAD	Nicotinamide adenine dinucleotide
ADP	Adenosine - 5' - diphosphate
AMP	Adenosine - 5' - monophosphate
GDP	Guanosine - 5' - diphosphate

INTRODUCTION

Two types of baking yeast are produced commercially, Compressed Yeast and Active Dry Yeast (ADY). The former contains 68-72% moisture and requires refrigerated storage for a three to five week shelf life. ADY is a dehydrated form of baking yeast and has a moisture content of 8%, which permits a shelf life of up to a year.

The development of ADY was greatly accelerated by World War II when Compressed Yeast could not be used conveniently by the armed forces in the field. Since then the use of ADY has extended to include domestic consumers and small bakeries where refrigerated storage facilities are not available. The task of developing a dehydrated yeast which retained its fermentative activity and exhibited extended storage stability at room temperature has continued for the past 40 years.

A variety of drying procedures and conditions for yeast have been reported in scientific and patent literature. Recently there has been reference to the importance of humidity conditions during ADY production. However, precise humidity levels at specified temperatures and drying rates have yet to emerge from patent literature. Much of the research contained in this thesis was performed to quantify these generalised comments and observations.

The precise levels of air humidity at specified temperatures and drying rates were determined for the optimum drying of Compressed Yeast. Activity was expressed as the leavening ability of dough, i.e. $mMCO_2$/hr/g yeast solid. Models were developed to describe yeast activity as a function of temperature, relative humidity and additive concentration.

The results of the drying experiments led to further investigations of Compressed Yeast production to increase the initial quality. A vast reservoir of literature has been published concerning Baker's Yeast. However, few authors have attempted to relate the effect of fermentation parameters on growing yeast cells to the final leavening ability. Research has centred around the use of static or standing yeast suspensions. The remaining experimental

research in this thesis was then spent identifying these key parameters.

The relationship between the accepted fermentation parameters for cell yield and those for leavening ability were compared and contrasted. The resulting discussion describes the role process parameters play in developing yeast fermentation character.

Throughout this thesis each chapter (Literature Review, Methods and Materials, Results and Discussion) is divided into three sections. Firstly that of Yeast Activity, which outlines the assay techniques developed to describe the CO_2 evolved in a dough. Secondly the Drying section outlines the investigations of producing Active Dry Yeast from Compressed Yeast. The third section describes the fermentation studies of media composition on yeast activity.

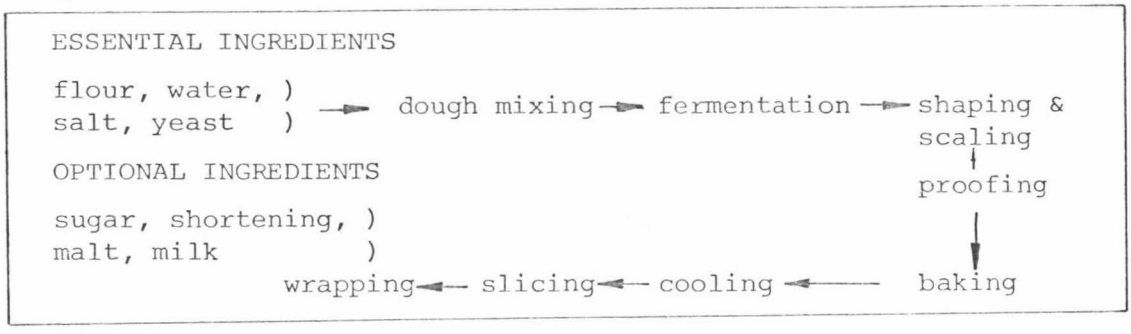
1. LITERATURE REVIEW

1.1 YEAST ACTIVITY

1.1.1 BREADMAKING

There is no record of when or where bread originated, but the history of bread runs parallel with the known history of man. A brief introduction to traditional breadmaking will be presented. This will, firstly preface a more detailed analysis of dough fermentation and secondly present dough fermentation within a perspective of other breadmaking procedures. The traditional process is outlined in Fig. 1.1.

Fig.1.1 Breadmaking



Dough refers to flour which has been moistened and kneaded while scaling is the dividing of the dough into pieces. Proofing is the final fermentation from the time the dough pieces have been placed in pans until they enter the oven. Generally proofing occurs at 28-32^{o}C. at high humidity for 0.5 to 1 hr (Pomeranz and Shellenberger, 1971; Pyler, 1973).

The basic ingredient of bread is flour, normally prepared from common wheat. Common New Zealand wheat varieties are Aotea, Kopara and Karamu (New Zealand Official Yearbook, 1979). Two new hybrid strains, expected to replace Karamu, are Rongotea and Oroua (McEwan et al., 1979). The breadmaking quality of flour depends upon the flour proteins, gliadin and glutenin, both of which are complex protein mixes (Aykroyd and Doughty, 1970). These water insoluble proteins form gluten when wetted and handled. During the fermentation and proofing, gluten is responsible for gas retention by formation of a skeleton or framework in the dough. Wheat flours are classified as either hard (or strong) i.e. those with a high percentage of protein (12%) or soft (or weak) i.e. flours with a low protein content

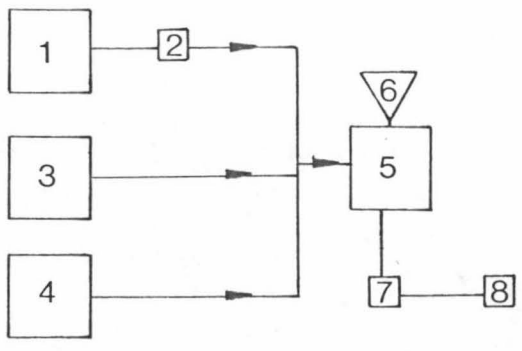
(7-9%) (Shellenberger, 1971). High protein flours produce excellent yeast leavened bread, while low protein flours are better suited for chemically leavened doughs. Other essential ingredients present are salt (approx. 1.5%) and yeast (approx. 2%) on a flour basis (Pomeranz and Shellenberger, 1971). The function of salt is to add flavour and colour, to strengthen and stabilize the gluten, and control yeast and bacterial activity.

Types of Fermentation

Several fermentation processes are used today. In a "straight fermentation" all ingredients (flour, liquids, sugar, salt, malt etc.) are mixed together, then rested for 2-2.5 hrs., before being punched three times at 50 min. intervals. This is more suited to low protein flours where leavening is dependent upon the amount of yeast (1.5-2%, on a flour basis). In a "sponge fermentation" a third of the total fluids and all the yeast, malt and a 1 : 1 ratio of flour and water are mixed and fermented for 20-30 min. The sponge is then remixed with the remaining ingredients and fermented for 1.5 hrs., and then punched twice. This process was developed from the use of Brewer's Yeast, before Baker's Yeast was available.

The "continuous process" originated in America in the 1950's e.g. the "Amflour" and "Do-maker" systems. The fermentable sugars are fermented in a "pre-ferment" for 2.5 hrs. by which the main liquids provide the necessary leavening power. Flour, up to 50%, may be added to the pre-ferment. A pre-mixer combines all the dough ingredients into a homogenous mass and a mixing unit develops, extrudes and scales the dough into individual pieces for immediate panning (Pyler, 1973). Fig. 1.2 illustrates the "Do-maker" System.

Fig. 1.2 The 'Do-maker' system



Key
(1) broth tanks
(2) heat exchanger
(3) oxidation solution tanks
(4) shortening tanks
(5) pre-mixer
(6) flour hopper
(7) dough pump
(8) developer & divider

From: Pyler (1973), Fig. 106, p. 685.

The amount of flour in the pre-ferment varies. A higher flour concentration gives a greater bread volume, a firmer texture and less mechanical energy is needed to develop the dough.

A typical final white bread composition is :

Moisture	35 - 45%
Carbohydrate	45 - 58%
Protein	6.0%
Fat	0.5 - 2%
Salt	1 - 1.5%

(Pomeranz and Shellenberger, 1971)

Fermentation does not stop at the end of the proofing period. When the dough is placed in the oven all the enzymic processes are greatly accelerated. As each critical temperature is reached, the enzyme reactions cease until the protein is finally coagulated. A great increase in dough volume occurs and is called "oven spring". This is caused partly by the thermal expansions of gases and vapours of the dough but also by fermentation. As the temperature inside a loaf reaches 50^{o}C. in 8-10 min., (Walden, 1955) fermentation action must occur within this period.

1.1.2 A Consideration of Dough leavening

Several enzymic and physiological systems operate in a fermenting dough. When flour is moistened, its physical structure begins to degrade, the extent of which is dependent upon the amount of milling the flour received and the amylase enzymes present. The products of degraded sugar must then pass across the yeast cell membrane (Plasmalemma) to be utilized. Consideration of sugar uptake and utilization is reviewed in section 1.3.4.

The Dough Components

The literature concerning starch is voluminous with extensive review articles (Anderson, 1946; Radley, 1976; Solomon, 1978). The starch molecule is an α - D(1$\rightarrow$4) linked glucan with α- D(1$\rightarrow$6) linked branched points i.e. a mixture of two polysaccharides. The major component of starch amylopectin, has a branched structure whilst the minor component amylose, is a linear molecule.

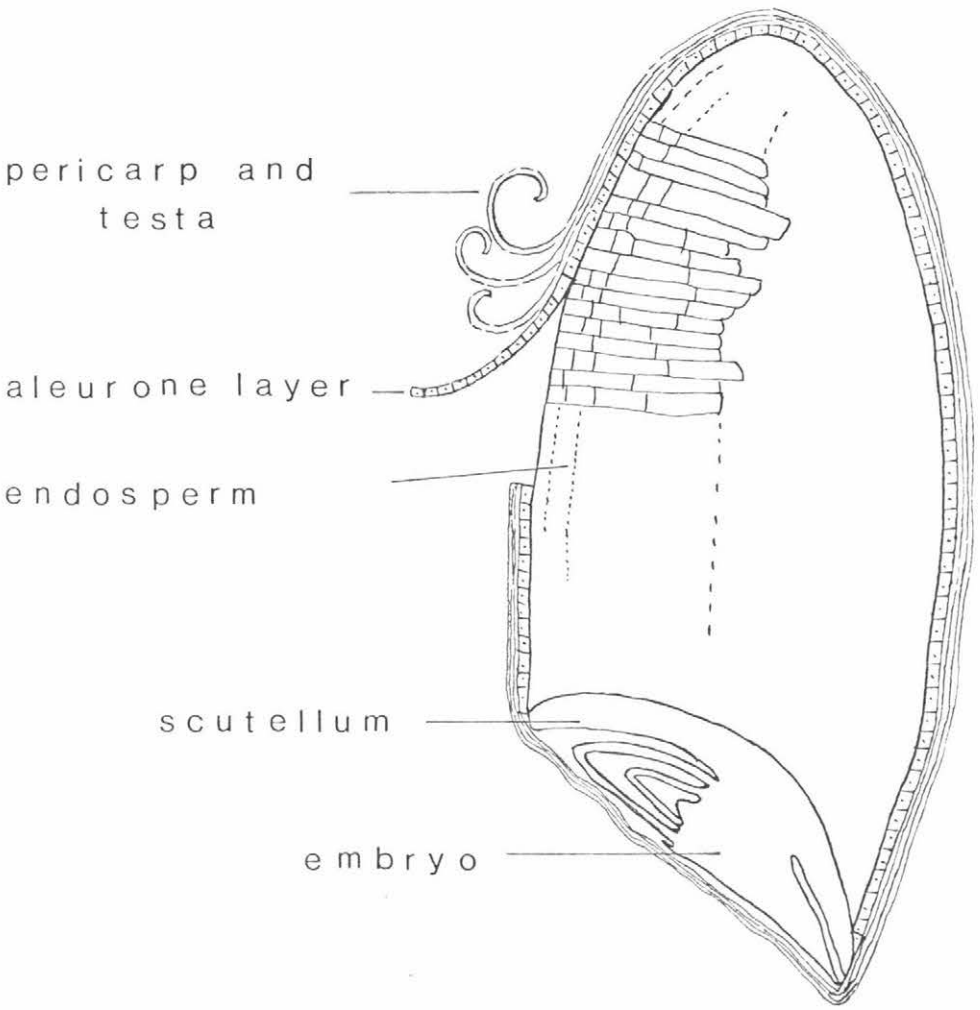
The group name originally given to enzymes catalyzing starch hydrolysis was diastase, later to be replaced by amylases. Amylases hydrolyze the α - 1, 4 - glycosidic linkage, most commonly being α or β amylase. α - Amylases are widely distributed in nature and split the bond in the interior of the substrate. Amylose degrades to maltose and maltotriose, while amylopectin also yields α - limit dextrins (oligosaccharides of four or more glucose residues containing α 1 - 6 glycosidic bonds). β - amylases hydrolyze from the non-reducing end of the substrate. Amylose is converted to maltose and maltotriose depending upon the number of D-glucose units present. Maltotriose is slowly hydrolyzed to D-glucose and maltose. Amylopectin is hydrolyzed like amylose except that the α- D (1→6) linkages remain in a limit dextrin. Typically, wheat has a high content of β - amylases and little α - amylase (Johnson and Miller, 1953). The maltose produced, up to 60% of the damaged starch granules, is available for yeast fermentation.

Moisture Adsorption by Dough

When flour is moistened, up to 36% water will be absorbed by the wheat starch when in a saturated atmosphere (water vapour at 21.5^{o}C.) (Radley, 1976). The distribution of starch granules in gluten varies with the wheat species, those embedded in the gluten are least accessible and absorb less water. Starch granules damaged by the milling process (2 - 4%) swell more than undamaged granules (Anderson, 1946). In wheat and barley amylases are centred in the scutellum of the embryo, the endosperm and aleurone layers with concentrations decreasing from the outer to the inner endosperm (Aykroyd and Doughty, 1970). Thus the endosperm, which has most starch, also has least amylase, see Fig. 1.3.

Damaged starch granules are more susceptible to amylase attack than unground flour. Milling brings the starch and amylase into more intimate contact and reduces the physical hardness of the endosperm. However, excessively overground flour leads to increased water uptake and storage instability (Radley, 1976).

Fig.1.3 Diagrammatic Longitudinal Section of Wheat Grain (Aykroyd and Doughty, 1970).



1.1.3 Yeast Activity Tests

The activity of Baker's Yeast can be evaluated by any method which permits the quantitative determination of the amount of CO_2 evolved, either by measuring its volume (at constant pressure) or its pressure (at constant volume). White (1954) classified yeast activity tests into methods using flour as the substrate and those which used fermentable sugars in a liquid medium as the substrate.

Tests Using Flour as the Substrate

An advantage of tests using flour as the substrate is that the fermentable sugars consist of a mixture of natural flour sugars in a natural medium. However, the properties of the flour used must be standardized (Burrows and Harrison, 1959).

The bake test:

The most commonly employed test in the baking industry is the Bake Test (Durham, 1965). It has been used in dough quality evaluation, yeast activity analysis, baking studies and product development. Although Bake tests represent the final criterion in dough quality, there are several associated problems.

First, a well equipped laboratory is required with temperature and humidity control facilities, mixers, proof cabinets, automatic moulders and large ovens (Reed and Peppler, 1973). Second, the human element can introduce up to 5% error (White, 1954). Third, there exists no standard Bake test. Durham (1965) stated "Although Cereal Laboratory Methods (AACC, 1962) described two standard bread baking tests, it is doubtful if any flour chemist employs either without modification. And so, after 50 years of experience and research, there is no generally accepted laboratory breadmaking formula and procedure". The range of processes and formulations can vary from 0 to 25% ($^{W}/_{W}$) sugar content, and the dough additives can include mould inhibitors, emulsifying and modifying agents or fermentation aids (Pyler, 1973). A Bake test, as described by White (1954), is listed on page 10 where the complexity of the test can be appreciated.

Measurement of gas production from a dough:

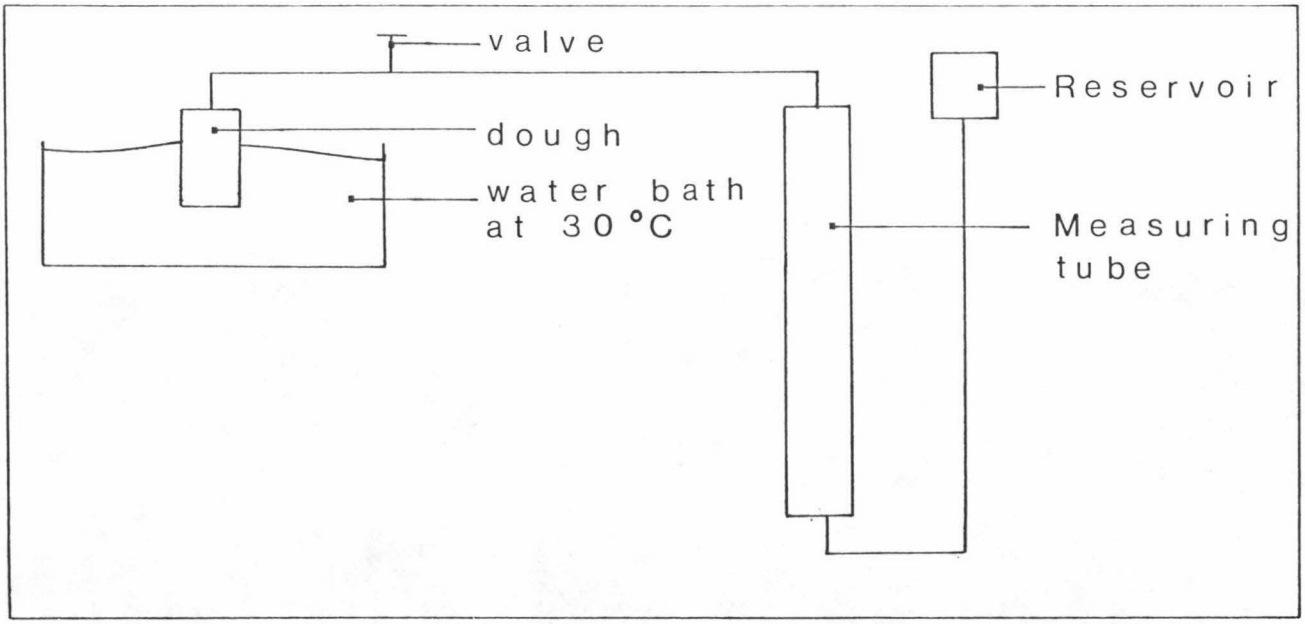
Basically, a dough is prepared and placed in a suitable container and the gas evolution or swelling of the dough is measured. Many devices

have been suggested.

Burrows and Harrison (1959) described a fermentometer consisting of flasks, containing a small volume of fermenting dough, in a water bath at 30°C. The gas evolved by the dough [flour plus a yeast-salt solution] was collected in a gas measuring burette. From the volume of displaced liquid [a solution of calcium chloride (2.7% W/V) and copper chloride (0.4% W/V)] , the evolved gas was measured. The pressure within the burette was adjusted to atmospheric pressure by a levelling reservoir for each reading. Fig. 1.4 illustrates this instrument. White (1954) suggested that a simple test would be to measure the expansion volume of dough in a measuring cylinder. This method does not appear to have been used elsewhere. Manometric methods measure the total gas production by the yeast i.e. the swelling of the dough plus the loss of gas through the dough.

An alternative instrument to Burrows and Harrison fermentometer was a constant volume manometer described by Hibberd and Parker (1976). The pressure required to keep a volume of dough constant, can be read from a pressure gauge. This apparatus is generally more complex than the constant pressure manometer. An apparatus designed to measure the gas volume produced, but not the dough expansion, by deflating the dough has been suggested (Voisey et al., 1964).

Fig.1.4 The Burrows and Harrison Fermentometer



A Standard Bake Test as quoted from White (1954).

"To be carried out in a room at 29°C.

5 g. of the yeast to be tested are weighed into a tared beaker and 156 ml. of a dilute sodium chloride solution containing 3.5 g. sodium chloride are added to produce a homogenous suspension of the yeast. The suspension is warmed to 29°C. and is gradually added to 280 g. of flour in a mixing machine. The flour is normally incubated in air-tight tins overnight at about 32°C; the temperature of the dough after mixing must always be arranged to be at 29°C. The flour and yeast suspension are then mixed for precisely 2.5 min. in the machine and the resulting dough is completely and expeditiously transferred into a standard baking tin (diameter 4 in., height 5 in.) and pressed down to a standard height by means of a stamping tool. This complete operation must take precisely 1 min. The tin is then put into the incubator at 34°C. The humidity of this "proof" cupboard should be 80-85%. The time taken for the dough to rise to a standard height in the tin (shown by a metal bar) is noted and this gives the first rising time. The dough is then mixed for precisely 0.5 min. in the mixer, replaced in the tin, stamped down and put back again into the incubator precisely as before. The time taken for the dough to reach the bar for the second rising is noted and the sum of the first and second rising times gives the baking test or fermentation efficiency of the yeast in the dough."

Nutrient solution as quoted by White (1954)

For 20 ml. vitamin - salt solution. pH adjusted to precisely 6.0 with H_2SO_4

Sodium dihydrogen phosphate	0.15 g.
Magnesium sulphate	0.08 g.
Potassium chloride	0.04 g.
Asparagine	0.4 g.
Aneurin (Vitamin B_1)	0.5 mg.
Pyridoxin (Vitamin B_6)	0.5 mg.
Nicotinic acid	1.6 mg.

Other instruments have been used to study dough, but were primarily developed for physical dough testing i.e. the influence of flour quality on the dough. These have been summarized by Brabender (1965). The most widely used recording dough mixer is the farinograph. This records the power needed to mix dough at a constant speed and provides information on the optimum mixing time, dough stability, water absorption and plasticity of a dough.

Tests Using Liquid Fermentable Sugars as the Substrate

Yeast activity tests based upon gas evolution during the fermentation of appropriate substrate solutions have been developed. The gas from reaction flasks continuously shaken in a water bath is measured directly with a gas burette. Schultz et al. (1942) described a nutrient solution fermentometer where flasks were shaken at 180-200 oscillations/min. at 30°C. The gas evolved was collected and measured in gasometers filled with 15% ($^{W}/_{V}$) sodium sulphate solution. The nutrient solution, containing potassium dihydrogen phosphate, yeast extract, fermentable sugar and the yeast under test (Shieh et al., 1973) can be regarded as a dough substitute. Another nutrient solution (White, 1954) is presented on page 10. The direct Warburg method has also been used for manometric measurement of yeast activity, at 30°C.

Gas Production as a Measure of Yeast Performance

It has been questioned whether the gassing rate of a yeast sample is a satisfactory measure of its performance (Reed and Peppler, 1973). Burrows and Harrison (1959), acknowledged that the relationship between dough volume and gas volume will be complicated by factors such as dough strength and gas diffusion. However, the authors stated that no yeast property except fermentation rate is responsible for any variable effect on dough structure and therefore on dough volume. Reed and Peppler (1973) believe this statement must be qualified as yeast vary greatly in their osmosensitivity. A gassing power test with a yeast/water/sugar solution will not reflect the activity of that yeast in dough which has a higher osmotic pressure. Thus, actual doughs rather than aqueous solutions should be used to determine the gassing power of yeasts.

At the same time the disadvantage of using standardized flour must be balanced against producing a synthetic medium for a complex fermentation reaction. Most test methods allow only a relative evaluation of one yeast sample against a standard yeast preparation. Standard samples often used are Active Dry Yeast, which is stored under nitrogen or vacuum (Thorn and Reed, 1959).

A Survey of Yeast Activity Testing Methods

As noted, there has been a variety of methods used to measure yeast activity. A survey of the procedures used by researchers in this area therefore seems appropriate.

It can be seen from Table 1.1 that of the yeast activity methods surveyed, nine were bake tests and eleven were manometric techniques. However, all of the bake test systems surveyed used differing formulae and triplicate tests were often carried out to reduce error. Manometric methods were split, either into procedures using a constant volume, or those using constant pressure apparatus. It would appear that a standardized approach to yeast activity will involve the use of manometric methods. The choice between constant volume/pressure is developed further in section 2.1.3 where a constant pressure system is proposed.

1.1.4 Factors Affecting Yeast Activity

During the course of the fermentation, yeasts bring about changes in the medium, mainly the depletion of fermentable substances, the accumulation of waste products and a modification of pH conditions. Dough fermentation is a complex system, even when additional variables such as the use of different flours, yeast strains and formulae are minimized.

Temperature

The effect of temperature on the gassing activity of yeast has been reported (White, 1954; Garver et al., 1966), but was related to specific dough and liquid substrates. Fermentation rate can be said to increase by a factor of 1.5 to 2 for each 10°C. increase in

Table 1.1 Yeast Activity Measurement by Various Authors

Method	Author	Research topic
Fermentometer	Biltcliffe, 1971	ADY fermentation of mono and disaccharides
Manometer	Blish & Sandstedt, 1937	Biocatalytic activators for maltose fermentation
Fermentometer	Burrows & Harrison, 1959	Method for measuring yeast activity
Bake test	Chen et al., 1966	ADY deterioration during storage
Bake test	Cimerman, 1966	Freeze-drying of ADY
Fermentometer	Ebbutt, 1961	Relationship between activity and cell wall permeability
Manometer, constant volume	Hibberd & Parker, 1976	Method for measuring yeast activity
Bake test	Felsher et al., 1955	Storage stability of ADY
Brabender farinograph, Bake test	Labuza et al., 1972	Effect of drying on cell viability
Fermentometer	Langejan, 1972	ADY by fluidized bed drying
Bake test	Mitchell & Enright, 1957	Effect of moisture on ADY
Manometer, constant volume	Morse & Fellers, 1949	Storage studies on ADY
Warburg respirometer Fermentometer	Oyass et al., 1948	Storage studies on ADY
Bake test	Peppler & Rudert, 1953	Methods for measuring yeast activity
Bake test	Pollock & Holmstrom, 1951	Relationship between trehalose content and quality of ADY
Bake test Farinograph	Ponte et al., 1960	Studies of ADY
Manometer, constant volume	Shogren et al., 1977	Determination of gas production
Manometer, constant volume	Thorn & Reed, 1959	Production of ADY
Automatic manometer	Voisey et al., 1964	Determination of gas production
Bake test	White, 1954	Studies of yeast

temperature to 45°C. (Reed and Peppler, 1973). Inactivation of yeast occurs between 54-56°C. (White, 1954).

Fermentable Sugars

In a dough, the initial enzymic activity is the yeast's fermentation of flour sugars. These are usually fructose, glucose, sucrose and maltose, a total concentration of 0.4% ($^{W}/W$) (Koch et al., 1954). The second enzymic activity is that of the amylases of the flour itself.

The subject of preferential utilization of sugar by yeast has been studied extensively (Blish and Sandstedt, 1937; Harris and Millin, 1962; Barnett, 1976). However, in breadmaking the fermentability of various oligosaccharides are not important (Reed and Peppler, 1973). Glucose, fructose and sucrose are rapidly fermented by Baker's Yeast, Fig. 1.5. Maltose is hydrolyzed to glucose by α - Glucosidase, part of an adaptive enzyme system (Lovgren and Hautera, 1977). The induction period, defined as the time before the maximal rate of maltose fermentation develops, varies according to the inducer. From Fig. 1.6, fermentation initiated by monosaccharides is sustained after 60 mins. by maltose fermentation. The biochemical utilization of sugar is reviewed in section 1.3.4.

pH

The maximal gassing activity is obtained between substrate pH values of 4 and 6 (Garver et al., 1966). Yeast fermentation has a broad optimum pH, compared with pure enzyme systems. This occurs as the internal pH of the cell (5.8) is quite constant regardless of pH variations in the dough or pre-ferment (White, 1954).

Osmotic Pressure

There is an increase in osmotic pressure in the dough caused by the high number of sugar and salt molecules. Yeasts vary in osmosensitivity, depending upon their production conditions. Partial inhibition of the rate of CO_2 production occurs at 1.5% salt (based on flour weight) (White and Munns, 1955). Commonly used dough salt levels are 2%. Thorn and Reed (1959) found an inverse relationship

Fig. 1.5 Sugar Depletion in Dough (Koch et al., 1954)

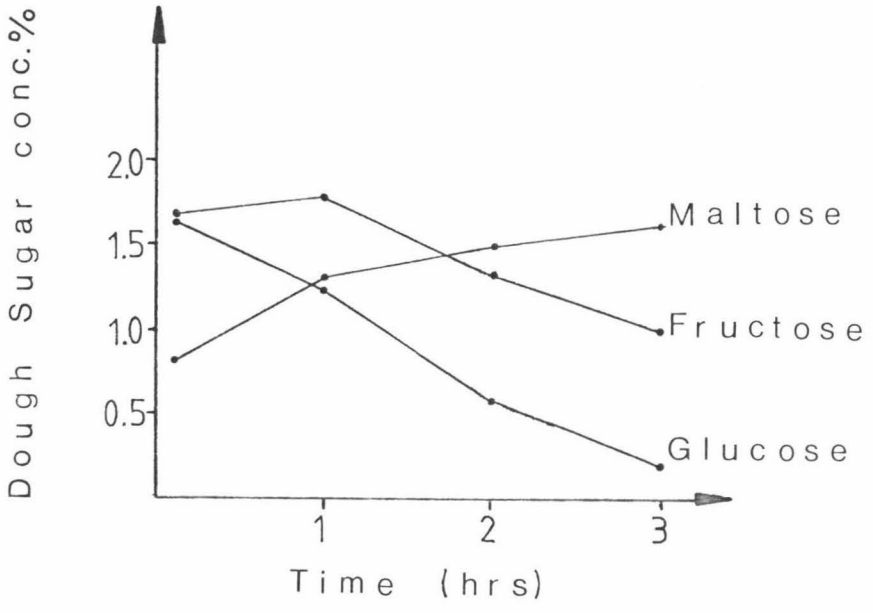
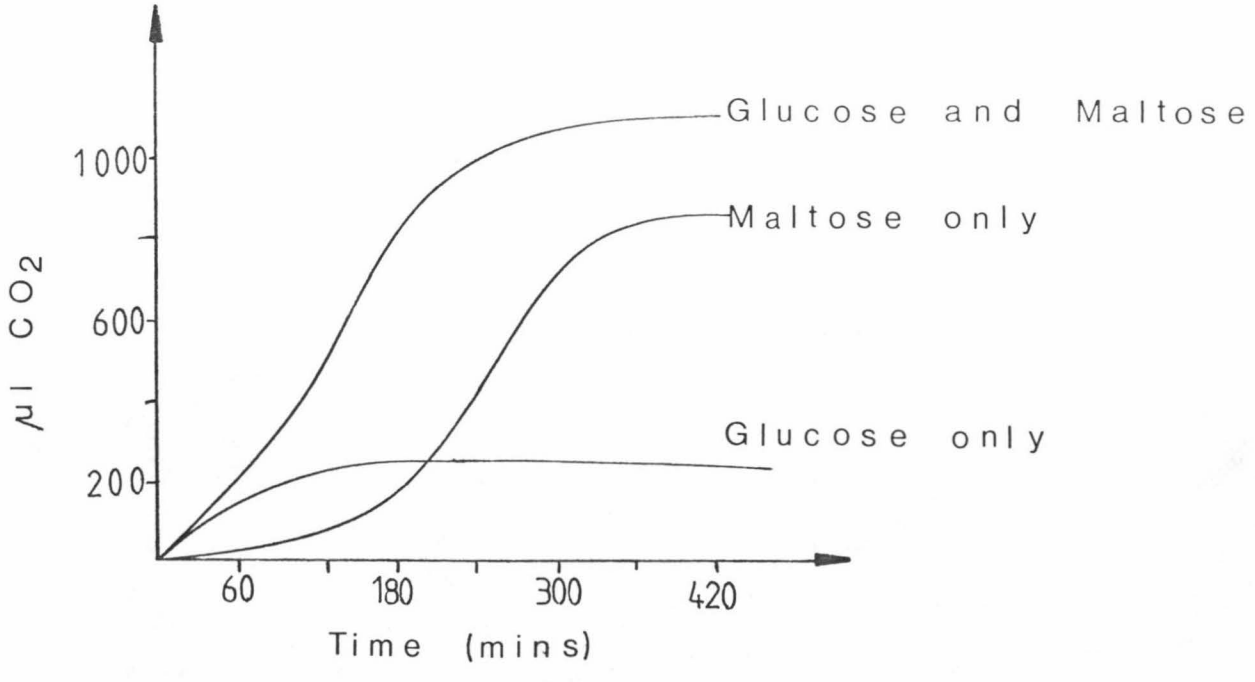


Fig. 1.6 Glucose and Maltose Fermentation by Baker's Yeast (Shieh et al., 1973)



between invertase activity of a yeast and its ability to ferment in the presence of 20-25% sugar (based on flour). In doughs, sugar concentrations up to 5% are not inhibitary (Pomeranz and Shellenberger, 1971). However, added sugar can range from 0 to 25%.

Storage

The activity of Compressed Yeast declines during storage (Phaff et al., 1978). Temperature is an important parameter and storage conditions ranging from +4°C. to -30°C. have been reported (Peppler, 1960). However, this range may not be the most practical for commercial distribution and storage. Some yeast is also distributed as "Crumbled Yeast" (Reed and Peppler, 1973). Filter cake is extruded through a perforated screen to produce irregular shaped pieces. These are packed into 22 kg. plastic lined paper bags.

Loss of activity during storage is associated with temperature increases of the product and endogenous respiration, resulting in the depletion of reserve carbohydrate i.e. glycogen and trehalose. Baker's Yeast usually contains 16-20% glycogen and 6-10% trehalose (Sols et al., 1970). Storage of ADY is reviewed (section 1.2.3) after a discussion of drying.

1.1.5 Rehydration

Before dried yeast can be used it must be restored to a hydrated and viable condition. Reconstitution is generally carried out by soaking the dried yeast in warm water at temperatures between 38°C. to 40°C. (Peppler and Rudert, 1953). If cold water is used, essential constituents leak from the cell and the fermentative activity of the yeast is reduced (Herrera et al., 1956). With ADY rehydrated at 40°C. a minimum (5% of the dry weight) of cellular material was leached (Ponte et al., 1960). Up to 25% of the soluble solids can be leached using cold water. Langejan (1972), detected the following components after leaching during rehydration: Nucleotides, NAD, NADH, ADP, AMP, GDP, and the amino acids glutamic acid, alanine and arginine. Methods have been developed to measure the extent of leakage (Chen and Peppler, 1956; Ebbutt, 1961).

Leached solids also affect the dough's rheology, making it more

relaxed and extensible. The slackening has been correlated with the presence of reduced glutathione in the leached solids (Ponte et al., 1960).

The rehydration procedures used by different authors are listed in Table 1.2. It can be seen that 40°C. (for 10-15 mins.) is an established rehydration procedure. However, the rehydration solutions vary in concentration and composition, as does the ratio of dried yeast to water. Before defining a procedure, for rehydration, the suspending agent requires further investigation.

1.2 Drying in Yeast Manufacture

The susceptibility of Compressed Yeast to spoilage has led to the development of Active Dry Yeast over a period of years. The production of ADY is an unusual process as it requires the survival of almost all the vegetative cells, without loss of viability.

1.2.1 Drying Theory

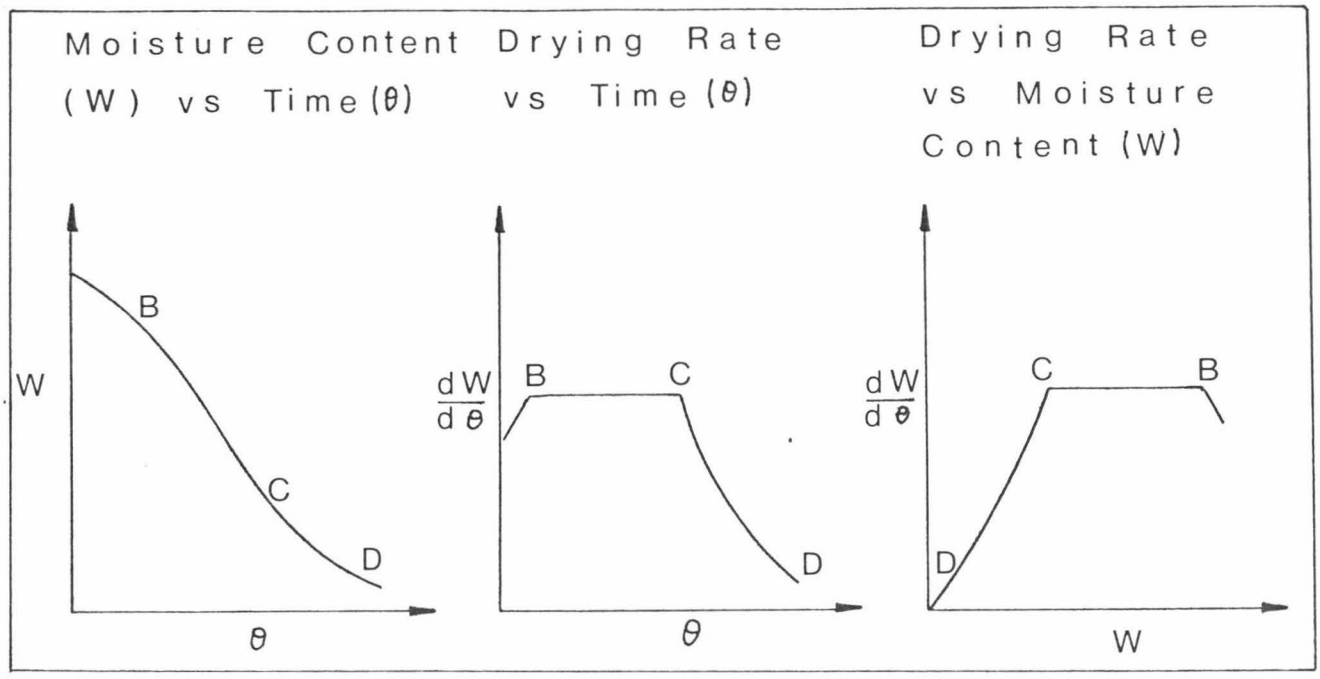
Removal of water during drying involves two mechanisms of heat and mass transfer. Firstly, when moisture is lying freely on the surface of the material being dried, the rate of removal is governed by the rate at which heat can be applied. Moisture removal is taking place at a constant rate. Secondly, when surface moisture has been removed another mechanism operates. The rate of drying becomes proportional to the rate at which moisture can diffuse by capillary action from inside the material. Drying is now taking place at a falling rate and the material's temperature approaches that of the heating medium (Bowmer, 1964).

Perry (1963), illustrated the drying periods as shown in Fig. 1.7 where sections B - C represent the constant-rate period, C - D represents the falling-rate period and point C is the critical moisture content.

Table 1.2 A selection of Rehydration Procedures for ADY

Procedure				Author	Research Topic
ratio ADY/solution	solution	time min.	temp. °C.		
1/66.7	water	15	37.5	Ebbutt, 1961	Activity and cell wall permeability
	2.5% NaCl	20	35	Cimerman, 1966	Freeze drying ADY
1/166	water	15	37.8	Biltcliffe, 1971	ADY fermentation of saccharides
1/13.5	3% sugar	15	40-43	Felsher, et al.,1955	Storage of ADY
1/10	water	2-3	43	Herrera, et al.,1956	Loss cell constituents after rehydration
Straight into dough				Labuza et al. 1972	Drying and cell viability
Straight into dough				Langejan, 1972	ADY fluidized bed drying
1/11.1	1% glucose	15	30	Oyass et al. 1948	ADY storage
1/19	water	10	37-43	Peppler & Rudert,1953	Measuring ADY activity
1/11.4	water	10	43.5	Pollock & Holmstrom, 1951	ADY and trehalose
1/20	water	20	40°C.	Ponte et al. 1960	Studies on ADY
1/4	water	10	40-45°C.	Thorn & Reed 1959	Production of ADY

Fig.1.7 Periods of Drying



At the critical moisture point (C), the rate of mass transfer across the liquid-gas interface, is in balance with the rate of mass transfer by diffusion and capillary action to the surface of the material. A mass balance over the constant rate period yields the relationship:

$$\frac{dW}{d\theta} = \frac{hc\ (t_d - t_s)}{h_{fg}}$$

where θ = time to dry, W = moisture content (dry weight basis), h_c = surface heat transfer coefficient, h_{fg} = latent heat of evaporation, t_d = air temperature, t_s = surface temperature of material or wet bulb temperature of air, t_w.

Below point C moisture supply to the surface is too slow to keep it fully wetted. Mass transfer through the solid now controls the drying rate.

For a more rigorous review of drying theory the reader is referred to Perry (1963), McCabe and Smith (1967) and Keey (1978).

1.2.2 Drying Processes

Preparation of Yeast Cream

Thorn and Reed (1959), reviewed the manufacture of ADY and listed conditions for what has developed as a standard preparation process.

Freshly propagated yeast is clarified, washed, and concentrated to a cream containing 14-18% yeast solids. It is then filter-pressed to form a cake (68-70% moisture content) and extruded through a perforated metal plate producing cylindrical threads of approximately 1.5 to 4.5 mm diameter. These threads are broken into sections varying from about 0.3 to 4 cm in length.

A number of processes for drying yeast cream is now considered.

Tunnel Drying

Continuous drying: Prepared yeast is packed on a continuous stainless steel open-mesh belt. Warm air, varying in temperature between 24°C. and 43°C., flows through the bed. Air humidity is regulated between 10-70% Hr to permit an equilibrium moisture content of 8% after 3 to 6 hours of operation (Thorn and Reed, 1959). In a four chamber drying tunnel, Reed and Peppler (1973) reported on air inlet temperatures of 42°, 37°, 32°, and 28°C. Generally, the air temperature does not exceed 45°C. for a yeast bed temperature of 40°C. The drying rate depends not only on air temperature and relative humidity but also on the air velocity (McCabe and Smith, 1967). Air velocities can range from 0.5 to 4 m/sec. Air humidity is frequently reported in literature as a design parameter quantified as a function of the outlet air temperature or as a change in humidity along the drying tunnel (Lapple et al., 1955; van Brakel, 1979). However, in recent patents describing ADY production there has been a greater appreciation of the importance of air humidity. For example, Taylor (1975)stated that relative humidity (Hr) should be less than 45%, preferably 35% depending on the temperature. A Russian report (Shishatskii et al., 1971) investigated aspects of dried yeast production. The "elevating strength" (minutes) reached a plateau between 20 to 35% Hr at 50°C. "Elevating strength" declined below 20% and increased slightly between 40 to 50% Hr. These authors concluded that temperatures between 30-50°C. held at 16-35% Hr are recommended for drying Baker's Yeast.

Batch drying: The yeast strands are layered on metal screens and then placed in drying chambers under conditions as described for continuous drying. The ADY obtained by this process has the characteristic shape of noodles with diameters, 2-3 mm and lengths 3-6 mm.

Fluid-Bed Drying

Extruded strands of yeast are deposited on a metal screen or perforated plate and air is blown through the yeast layer, thus suspending the yeast in a fluid bed. Langejan (1972) reported much reduced drying times, 10 to 30 mins. The initial drying air temperatures ranged from 100°C. to 150°C. and were adjusted to keep the yeast particles in a 25°C. temperature range. Lower air temperatures, between 30°C. to 50°C., have also been reported (Taylor, 1975).

Rotolouvre Drying

Semi-fluidized bed conditions are created by feeding extruded yeast strands into a rotating metal cylinder. Warm air is blown through louvres into the cylinder and upwards through the yeast bed. Typical conditions are yeast temperatures not exceeding 45°C., and drying times between 10 to 20 hrs. Dry yeast pellets are typically about 2 mm diameter. These pellets are more stable on storage, probably due to the smaller surface area per weight of yeast and their smooth surface (Thorn and Reed, 1959).

Lyophilization

Freeze-drying has long been used for the preservation of yeast cultures, but can result in a considerable loss of viability (Atkin et al., 1962). If few yeast cells do survive, there is a possibility of selecting mutants, as observed by Wynants (1962), with unstable fermentation characteristics (Scheda and Yarrow, 1966). Further, the viability of a culture may be a poor index to its fermentative character (Mitchell and Enright, 1957).

An optimum cooling rate for maximum survival of Saccharomyces cerevisiae has been determined. Mazur (1970) first reported this as 7°C/min. A widely used cooling condition is 1°C/min. (Heckly, 1978), as there is no clear cut optimum. When cooling at rates slower than 1°C/min., solute effects are responsible for injury. However at faster cooling rates, the injury is related to intracellular freezing (Mazur, 1970).

Drying should take place below a critical temperature, at which recrystallization will occur. If rapid cooling is used, ice formed

in the cells would be likely to grow should any warming occur, especially slow warming (Mazur and Schmidt, 1968).

Cryoprotective agents e.g. glycerol, polyvinylpyrrolidone (PVP), dimethyl sulfoxide (DMSO) and sugars have been used to protect cells against injury. A mixture of 10% glycerol with 5% lactose, maltose or raffinose has been suggested for S.cerevisiae (Daily and Higgens, 1973).

The impression one is left with after consulting the literature is that freeze-drying is not successful for the commercial preparation of Active Dry Yeast. At the optimum cooling conditions cited by Mazur and Schmidt, (1968) and Mazur, (1970) the cell survival was still only 10%.

1.2.3 Dried Yeast Storage

After drying, Active Dry Yeast has a much greater stability than Compressed Yeast. The activity of ADY can be further extended, depending upon the storage conditions.

Active Dry Yeast loses 7% of its activity over a period of a month at conditions prevailing in bake shops i.e. relatively high temperatures and packages open to the atmosphere. (Thorn and Reed, 1959). Under conditions of relatively low temperatures and packed in vacuum or under nitrogen gas, the loss of activity over one year was approximately 10% (Reed and Peppler, 1973). Loss of activity is greatly accelerated by the presence of oxygen. Dried yeast has extended storage times when stored in atmospheres of carbon dioxide, nitrogen or in vacuo (Oyass et al., 1948). Of the following storage factors, packaging, atmosphere, light, time and temperature, Morse and Fellers (1949) found that temperature had the greatest influence on viability of ADY. This was also verified by Felsher et al., (1955), where ADY stored at 4.4^{o}C. was still viable after 2 years.

Various techniques have been investigated to increase ADY storage time. Mitchell and Enright (1957) found low moisture level (between 2.5 and 5.5%) ADY preparations were more thermostable than regular commercial ADY of 8% moisture. However, vapour-rehydration to 8% was required to avoid leaching of intra-cellular polymers e.g. NAD &

AMP (see section 1.1.5). Storage time can be increased by the incorporation of certain antioxidant substances into the yeast slurry prior to drying to 4-6% moisture. Such products are referred to as "protected ADY" (Chen et al., 1966). Protected ADY had the equivalent storage viability as ADY stored under nitrogen or vacuum. Butylated hydroxyanisole (o.3%) was the most effective antioxidant, and sorbiton monostearate (2%) was an effective emulsifying agent (Chen et al., 1966). Emulsifiers reduce cell components leaching during rehydration and antioxidants protect against the effects of atmospheric oxygen. However the protective effect of the antioxidant cannot be demonstrated at yeast moisture levels above 7.5% to 8.5%. (Reed and Peppler, 1973). The emulsifier plus antioxidant formula used is listed in Table 2.3.

ADY for consumer packs (7g) are packed under nitrogen in envelopes consisting of an inner layer of polyethylene and an outer layer of Aluminium foil, which is then heat sealed (Burrows, 1970).

1.2.4 Isotherm Studies

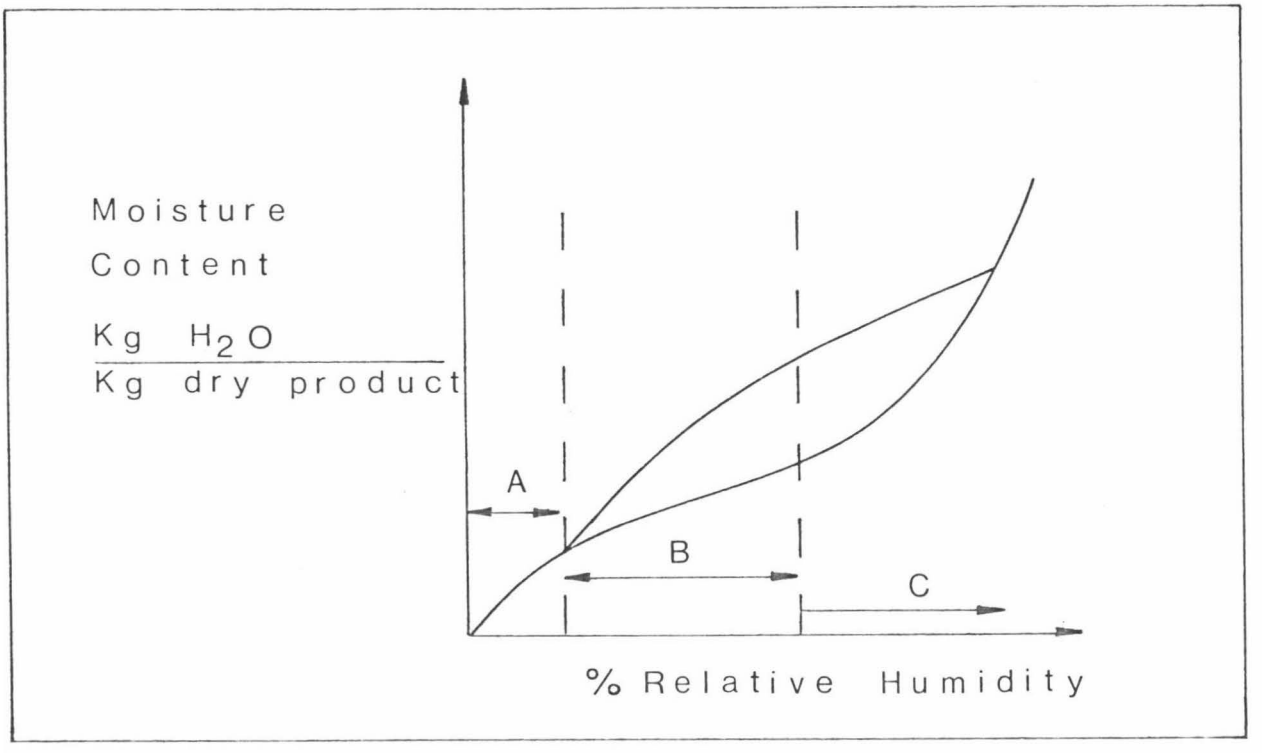
Moisture sorption isotherms show in graphical form the variation in moisture content of a sample with the Equilibrium Relative Humidity (ERH) at a specified temperature. A general sorption isotherm, as discussed by Labuza (1968) is presented in Fig. 1.8. Region A of Fig. 1.8 corresponds to the adsorption of a monomolecular film of water, Region B to adsorption of additional layers over the monolayer, and Region C to condensation of water in the pores of the material, followed by dissolution of the soluble material present. Salwin (1959) has shown the monolayer value (Region A) to be the most stable water content for most foods.

Several mathematical models have been proposed to describe isotherms. These have been comprehensively reviewed by Salwin (1963), Labuza (1968), Clifford (1975). The usefulness of any theoretical approach will depend on the objectives of the user. Three types of approaches have been made, the kinetic, the potential and the capillary condensation models. Of these, the capillary condensation approach has had limited application in the food field as the theory is designed to apply to high relative humidities. Likewise, potential models

have found little application to food systems as they do not allow an estimation of the monolayer capacity. Kinetic models are frequently used to describe foods and of these the Brunauer, Emmett and Teller (1938)(BET) is predominant. From it the monolayer coverage of water can be calculated.

One further approach is the use of empirical relationships to describe sorption isotherms. Boquet et al, (1978) surveyed two-parameter equations for sorption isotherms and found that the Oswin (1946) equation gave a good fit to many food types.

Fig. I.8 General Sorption Isotherm



1.3 Fermentation of Baker's Yeast

Baker's Yeast fermentation and key parameters in the process will be reviewed. The role nutrients and sugars play in fermentation, together with key metabolic regulators of Baker's Yeast, will be outlined. It is not proposed to review the areas of cell structure and form, nor that of growth principles. These areas were deemed to be outside the range of research undertaken.

1.3.1 An Outline of Baker's Yeast Production

The production of yeast specifically for bread-making has been a manufacturing operation since the early 19th century. During the course of time three major process developments have occurred, these being the use of aeration, incremental substrate feeding and use of molasses as the carbon source for yeast growth. With the introduction of the 'Vienna' process in the 1860's and from the well known research of Pasteur, the yields of yeast were substantially increased. Between 1915 and 1920 incremental feeding was developed (the Zulauf process). This technique avoids the production of large quantities of alcohol associated with earlier methods. The final development was the replacement of the original saccharified grain substrate of corn and malt with less expensive molasses sugar. Molasses was supplemented with phosphate and ammonia to form the growth medium. These changes formed the basis of modern Baker's Yeast production throughout the world.

The Manufacturing Process

In 1977 the world production of Baker's Yeast (excluding U.S.S.R.), was 187,700 tonnes (dry weight) (Peppler, 1978). This was produced by conventional fermentation procedures, as outlined below.

The raw material for Baker's Yeast production, beet or cane molasses, is incrementally added to the fermenting yeast (Rosen, 1968). The composition, supplements and treatment of molasses is considered in section 1.3.3 on yeast nutrition. Large quantities of yeast are required as inocula for the final propagation stage. From pure cultures the yeast seed passes through many stages of increasing volume. Four stages are common and rarely does propagation go beyond six, due to contamination (White, 1954). Fermentation conditions vary during the sequence of propagation (Burrows, 1970), however it is the final production stage that determines the yeast's properties.

A Baker's yeast fermentation is aerated with 0.5 to 1 volume of air per fermenter volume per minute and agitated at between 300-400 r.p.m. An eleven hour fermentation with molasses fed exponentially is common, and finishing with an hour for maturation and ripening. This

results in yeast multiplying four to five times with final yields varying between 3.5% and 4.5% solids or 5 g dry matter / 10 g glucose (Reid, 1969; Solomons,1969; Burrows, 1970; Phaff et al., 1978). The optimum balance of properties is determined by accurate control of nutrient feed rates, temperature, pH levels and aeration. With modern measuring devices currently available, fermentation control can now be interfaced with micro-processors allowing automatic process control (Ryu and Humphrey, 1973; Wang, 1977; Rosen, 1977).

1.3.2 Fermentation Parameters

pH

Best yields of Baker's Yeast occur between pH values of 4.5 and 5.0 and the range for growth can extend from 3.5 to 7.0 (Phaff et al., 1978). During fermentation low pH will minimise the risk of contamination, while at higher pH values less molasses pigments are adsorbed onto the yeast (White, 1954). Using liquid preferments for continuous breadmaking (i.e. standing yeast cells) Garver et al. (1966) reported an increase in CO_2 evolved with decreasing pH to a maximum at pH 4.0. However, the influence of fermentation pH on loaf volume was exactly the opposite. It was suggested that pH must influence other factors, such as CO_2 retention in the dough, which overrides its effect on yeast activity.

Temperature

Yeast growth will occur between 20-40^{o}C. (White and Munn, 1951), though the commercial range is restricted to 25-35^{o}C. The temperature for the optimal growth rate of yeast (35^{o}C.) and CO_2 evolution (37^{o}C.) differed from that for cell yield (30^{o}C.) using standing cells (Merritt, 1966). In bacteria this phenomenon has been associated with the greater availability of oxygen at lower temperatures (Sinclair and Stokes, 1963). Temperature adaption and special nutrients are thought to be necessary for yeast growth at elevated temperatures (40^{o}C.) (Stokes, 1971).

Oxygen

Although limited growth of yeast occurs under anaerobic conditions, oxygen is required for maximum growth and the efficient utilization

of substrate (Harrison, 1967). The amount of oxygen required can be calculated theoretically e.g. if 100 g of sucrose yields 50 g of yeast solids then 1 g oxygen per g yeast solids is required. Harrison (1967) calculated 1.025 g oxygen would be required for the growth of 1 g yeast solids. As the solubility of oxygen in water is low, and the rate of removal by growing yeast is high, oxygen limits the rate of yeast growth. This has lead to a variety of sparging and agitation systems which attempt to increase the rate of mass transfer of oxygen to the fermentation broth (Solomons, 1969). The metabolic regulatory effect that oxygen has on the yeast cell undergoing fermentation is reviewed in section 1.3.5.

Yeast Strain

Pure strains of Saccharomyces cerevisiae are almost universally employed in producing Baker's Yeast. Attempts to produce new strains, possessing better qualities, continue through hybridisation (Rosen, 1968). Langejan (1972) reported yeast hybrids for ADY production, however none have come into general use (Peppler, 1979). ADY is generally produced with a strain typified by strain No. 7752 in the American Type Culture Collection (Reed and Peppler, 1973). This strain is grown under nitrogen limitation, and generally does not exceed cell levels of 6.5 to 7.5% nitrogen. In comparison, Compressed Yeast reaches levels of 9% nitrogen. Nitrogen restriction results in an increased total carbohydrate content in the cell, particularly trehalose, which appears to confer stability during the drying process (Pollock and Holmstrom, 1951).

Assimilation and Budding

The final hour of fermentation is usually deemed a "ripening" period with no feed and little aeration (White, 1954). Unused nutrients are assimilated, budding cells divide and mature, thus synchronizing their reproductive mechanisms for the beginning of the next budding cycle. During budding the yeast takes up considerable quantities of phosphorus from the medium, but little nitrogen (Suomalainen, 1975). The carbohydrate content, especially glycogen and trehalose, also increases.

1.3.3 Yeast Nutrition

Carbon

The growth substrate is one further parameter influencing the fermentation process. Saccharomyces cerevisiae will metabolize a wide range of sugars e.g. glucose, fructose, maltose and sucrose (Lodder and Kreger van Rij, 1967). Molasses, a by-product of the sugar industry, is an inexpensive source of sugar as well as having a high concentration of utilizable sugars (Patarau, 1969). The composition of molasses produced from beet and cane sugar is listed in Table 1.3.

Table 1.3 Molasses Composition (White, 1954)

Component	Beet	Cane
% reducing sugar (as invert)	48-58	50-58
% N_2	0.2-2.8	0.08-0.5
% P_2O_5	0.02-0.07	0.01-0.07
% ash	4.0-8.0	3.5-7.5
% CaO	0.15-0.7	0.15-0.8
% K_2O	2.2-4.5	0.8-2.2
% MgO	0.01-0.1	0.25-0.8

Beet molasses is generally higher in nitrogen and potassium than cane molasses. Molasses supplies all trace metal requirements for yeast growth, but is deficient in phosphate (Burrows, 1971). This can be added as ammonium phosphate.

Prior to its use, molasses requires two major purification treatments. Firstly, sterilization by a high temperature short time process and secondly, centrifugation to remove sludge (Reid, 1969).

Nitrogen

The Gay-Lussac equation for the fermentation of glucose was expanded by Harrison (1967) into an empirical formula to include nitrogen (based on 200 g glucose).

$$0.585\ C_{12}H_{22}O_{11} + 0.61\ NH_3 + 3.205\ O_2$$

$$\longrightarrow C_{3.72}H_{6.11}O_{1.95}N_{0.61} + 4.29\ H_2O + 3.3\ CO_2$$

Nitrogen is added in the form of ammonia or ammonium salts (Rosen, 1968). The acidifying effect of assimilating ammonium sulphate can be used along with ammonia for two way pH control.

Thorne (1954) reported, when using resting cells, an increasing fermentation rate (CO_2 evolved) with increasing nitrogen levels of Compressed Yeast. However low nitrogen levels (6-7% of dry matter) together with a high carbohydrate content has been associated with dried yeast manufacture (Peppler, 1979).

Vitamins and Minerals

Yeast requires several vitamins for growth. For example Baker's Yeast requires 1 ppm of biotin (based on dry weight) or alternatively L (+) aspartic acid together with oleic acid (Suomalainen and Keranen, 1963).

Synthetic Media

To ensure there are no variations in nutrients, a synthetic medium is often used for growth experiments. It contains a number of trace elements, vitamins, amino-acids as well as principal minerals e.g. potassium, phosphate and magnesium sulphate. The medium Olson and Johnson (1949) developed (as listed below) was a significant advance in the study of optimal medium composition. A yield of 100 g yeast dry matter / 250 g glucose was obtained. McMurrough and Rose (1967) also obtained a yield of 100 g / 250 g glucose. Oura (1974) obtained 100 g / 193 g glucose. The theoretical yield was 100 g yeast / 200 g glucose.

A Synthetic Media, as quoted by Olson and Johnson (1949).

Glucose	10.0 g per litre
$NH_4H_2PO_4$	6.0 g
KH_2PO_4	0.2 g
$MgSO_4 \cdot 7H_2O$	0.25g
Sodium citrate	1.0 g
L- Asparagine	2.5 g
Biotin	20 µg
Calcium pantothenate	0.5mg
Inositol	10 mg
Thiamine	4 mg
Pyridoxine	1 mg
Zinc (as sulphate)	400 µg
Iron (as ferrous ammonium sulphate)	150 µg
Copper (as sulphate)	25 µg
Water to 1,000 ml	
PH adjusted to 5.0 with H_2PO_4	

1.3.4 Sugar Utilization

The biochemistry of sugar utilization and the related cell regulatory mechanisms will be discussed. This will form the basis for considering the relationship between leavening ability of Compressed Yeast and the fermentation conditions during production.

Sugar Uptake

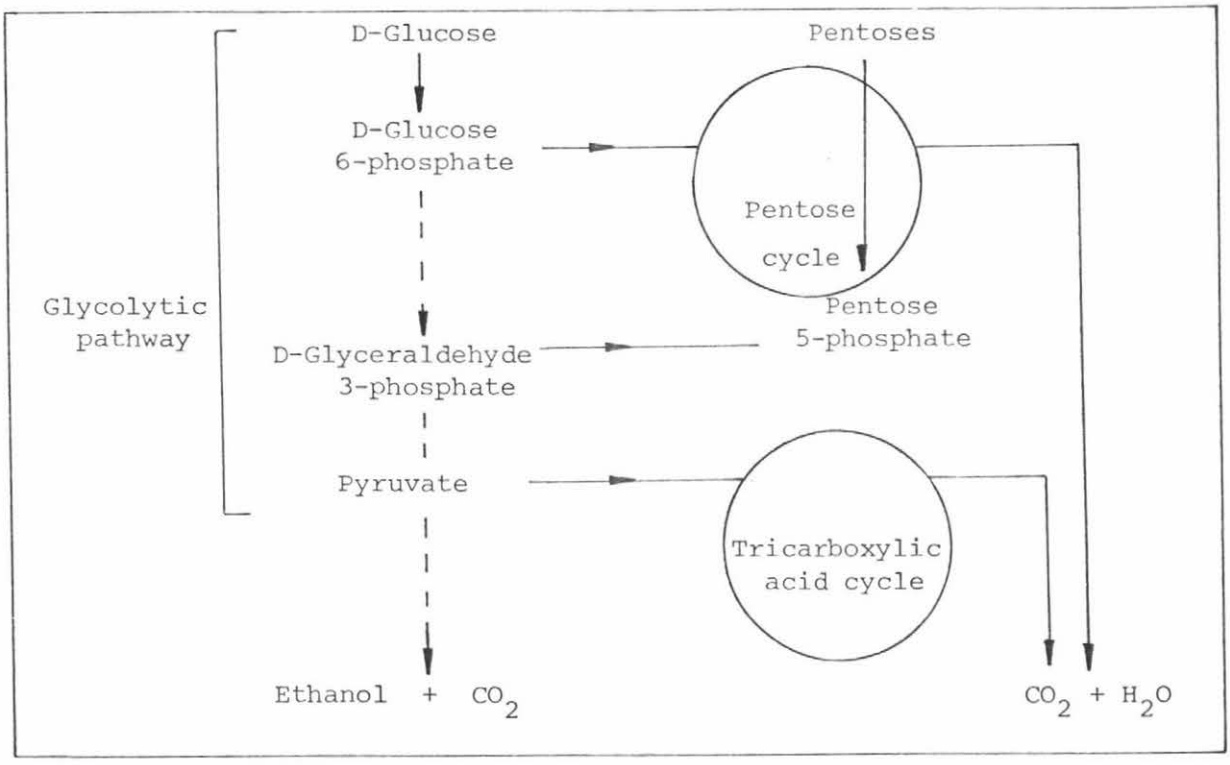
The first step in sugar utilization is the movement of sugar across the plasmalemma, usually with the aid of a carrier. These are similar to enzymes in that they are proteins, form complexes with their substrate, have varying degrees of specificity and are inducible and repressible (Barnett, 1976). Movement can be either facilitated diffusion, where no metabolic energy is required, or active transport where metabolic energy is expended. The topic of sugar transport was reviewed by Suomalainen and Oura (1971) and Jennings (1974). The number and nature of carriers used in active transport is still controversial. Barnett (1976) listed ten qualifiers when considering mechanisms of sugar transport. Differ-

ences in opinion concerning sugar uptake may be related to the techniques and strains of yeast used by different authors.

Sugar Metabolism

The intermediary metabolism of carbohydrates has been described in standard textbooks of Biochemistry (White et al., 1973; Conn and Stumpf, 1976). The main steps are presented here to show the interrelationships of the central pathways of carbohydrate catabolism in yeast (see Fig. 1.9).

Fig. 1.9 Interrelationship of the Central Pathways of Carbohydrate Catabolism in Yeasts (Barnett, 1976)



Anaerobic Catabolism of D-glucose: In the anaerobic fermentation of sugar, yeasts convert the pyruvate produced by glycolysis into ethanol by way of acetaldehyde. The overall equation for alcoholic fermentation was established by Gay-Lussac as:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Aerobic Catabolism of D-glucose: Under aerobic conditions yeasts convert pyruvate produced by glycolysis into acetylcoenzyme A. This is the starting point for the tricarboxylic acid cycle (T.C.A). During the T.C.A. cycle carbohydrates are oxidized to carbon dioxide

and water, and intermediates of the cycle serve as precursors for the synthesis of many cellular constituents. D-glucose may also be catabolized by the pentose phosphate cycle (White et al., 1973). The pentose phosphate cycle contains two reactions capable of producing the reductant NADPH as well as a number of different sugar phosphates.

Chen (1959) reported that during aerobic fermentation (69 mmoles O_2 / ℓ / hr) with actively growing yeast, 60% of glucose carbon was assimilated into the yeast cell and 40% was metabolized to CO_2. Of the total CO_2 produced, 51% was derived from the decarboxylation of pyruvate, 43% from the T.C.A.-oxidative pathway and 6% via the hexose monophosphate shunt. Reservations have been expressed concerning radiorespirometric techniques, (Axelrod, 1967) used to derive these results. The fraction of glucose catabolized by the pentose-phosphate pathway would, however, appear to be less than 15% (Wang et al., 1958; Katz and Wood, 1963).

After considering sugar utilization in Baker's Yeast, there appears to be three reasons why sugar will fail to be metabolized. Firstly when sugar does not enter the cell; secondly when the yeast lacks one or more enzymes necessary to convert the substrate into an intermediate metabolite of a central pathway; and thirdly when the central pathway itself is inoperative from lack of an essential enzyme.

1.3.5 Metabolic Regulation

Glucose

Much has been published on the controversial subject of control of glycolysis since the observations of Pasteur (1876) and Crabtree (1929). The following summary of catabolite repression is based on the reviews of Sols et al. (1971), Barnett (1976) and Phaff et al. (1978).

Isocitrate dehydrogenase (NAD^+), which catalyses the T C A cycle reaction:

$$\text{isocitrate} + NAD^+ \longrightarrow \alpha\text{- ketoglutarate} + CO_2 + NADH + H^+$$

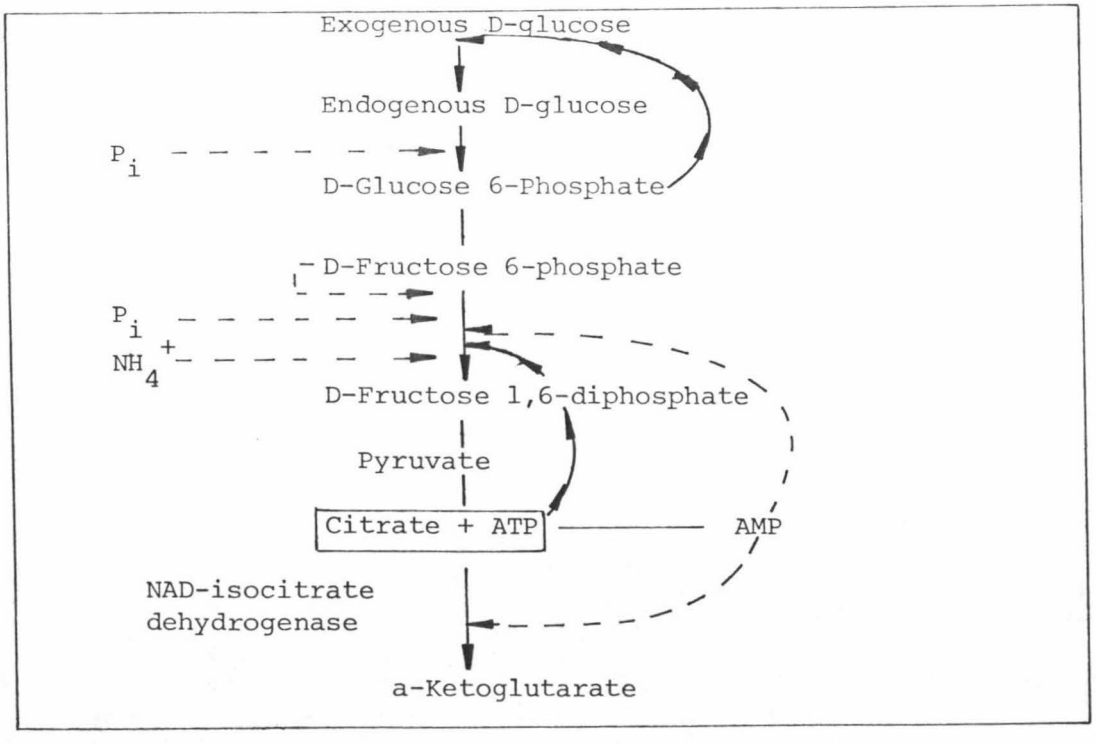
is dependent on allosteric activation by AMP (Hathaway and Atkinson, 1963). When the AMP/ATP ratio is low, the citrate concentration increases (see Fig. 1.10).

6-Phosphofructokinase, responsible for phosphorylating D-fructose 6-phosphate to D-fructose 1, 6-diphosphate is inhibited allosterically by citrate and ATP, and activated by AMP (Sols, 1968). This leads to a build-up of D-glucose-6-phosphate. That in turn has been suggested (Sols, 1968) to reduce D-glucose transport across the plasmalemma (see Fig. 1.10).

Thus glucose has a detrimental effect on respiration i.e. catabolite repression (Gorts, 1967). Biosynthesis of these enzymes is prevented because the end-product activates a latent repressor that subsequently switches off the appropriate operon (Wiseman, 1975). When the cell is rich in ATP the biosynthesis of the respiration enzymes is repressed. De-repression of enzyme biosynthesis occurs when the end product, or its controlling influence, is removed.

In a Baker's Yeast fermentation, incremental feeding of glucose will avoid the repression of respiratory enzymes. Thus the Compressed Yeast produced will have a high respiratory activity.

Fig.1.10 Control of Glycolysis (Points of repression indicated by arrows, and of activation by dotted lines, Barnett, 1976)



The actual glucose concentrations used in commercial Baker's

Yeast fermentation are kept below 0.1%. Though Markham (1969) reported a maximum of 3% glucose, Phaff et al. (1978) and Peppler (1979) suggested that glucose concentrations should be less than 0.1 %.

Repression of the maltose uptake system in yeast also occurs in a medium containing glucose or fructose (Lovgren and Hautera, 1977). Little maltose is utilized until the concentration of monosaccharides falls below 0.2% and a derepression of the maltose uptake system occurs. However, no correlation has been found between the α - glucosidase activity (maltase) and the leavening power, nor the ability to ferment maltose (Suomalainen, 1975).

Oxygen

Pasteur was the first to demonstrate that a Saccharomyces yeast, growing on low concentrations of glucose, decreased its fermentative activity when subjected to aeration. Part of the glucose was respired to carbon dioxide and water. The change in free energy for anaerobic conversion of D-glucose into ethanol is given by the equation:

$$C_6H_{12}O_6 \longrightarrow 2EtOH + 2CO_2 \qquad G' = -235 \text{ KJ}$$

and aerobic oxidation (Krebs et al., 1957) by:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad G' = -2{,}873 \text{ KJ}.$$

Thus, a decrease in the consumption of D-glucose occurs, with respect to cell yield when changing from anaerobic to aerobic conditions.

Oxygen will also act as a controlling mechanism for the yeast cell. This is apparent when the glucose uptake rate is low (1.2 - 2.8 mmoles / hr / g yeast solid) (Moss et al., 1971). When glucose is absent oxygen inhibits the formation of alcohol dehydrogenase enzymes, of which the yeast cell has three (Oura, 1976). Using a wholly oxidative metabolism, the available oxygen is the main agent controlling the ability of the yeast to support growth (Oura, 1974). If the oxygen demand is in excess of the rate at which it can be supplied, anaerobic fermentation will occur. In Baker's yeast this can be avoided if another nutrient is set at a limiting concentration (Markham, 1969). The yeast would then develop its full respiratory capacity.

Applications of Metabolic Control

It can be seen from the literature review, that the influence fermentation parameters have on cell yield has been well documented (see section 1.3.2). Their role concerning the functional properties of the yeast, such as leavening ability, has received much less coverage (see section 1.1.4). However, a knowledge of the yeast's metabolic regulation will provide a basis for interpreting fermentation data and relating it to the leavening capacity.