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.

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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 $40^{o}C$. 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 $20^{o}C$.

Fermentation parameters were correlated with the activity of Compressed Yeast using an experimental design. Growth temperatures varied from $28^{o}C$. to $37^{o}C$., 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, $28^{o}C$. 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.

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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
$\Delta G'$	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.

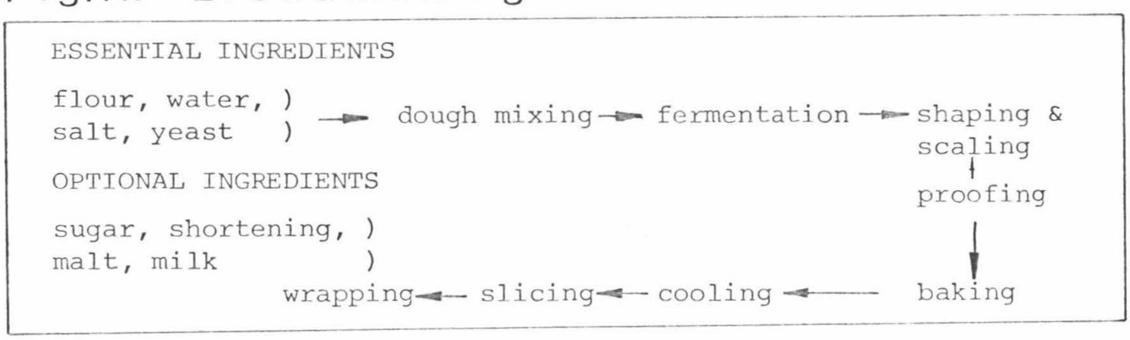
1. LITERATURE REVIEW

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

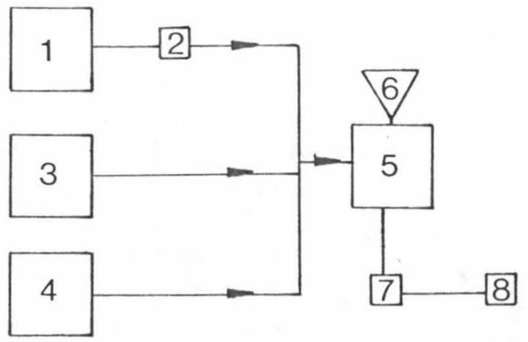
(7-9%) (Shellenberger, 1971). High protein flours produce excellent yeast leavened bread, while low protein flours are better suited for chemically leavened doughs. Other essential ingredients present are salt (approx. 1.5%) and yeast (approx. 2%) on a flour basis (Pomeranz and Shellenberger, 1971). 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°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.

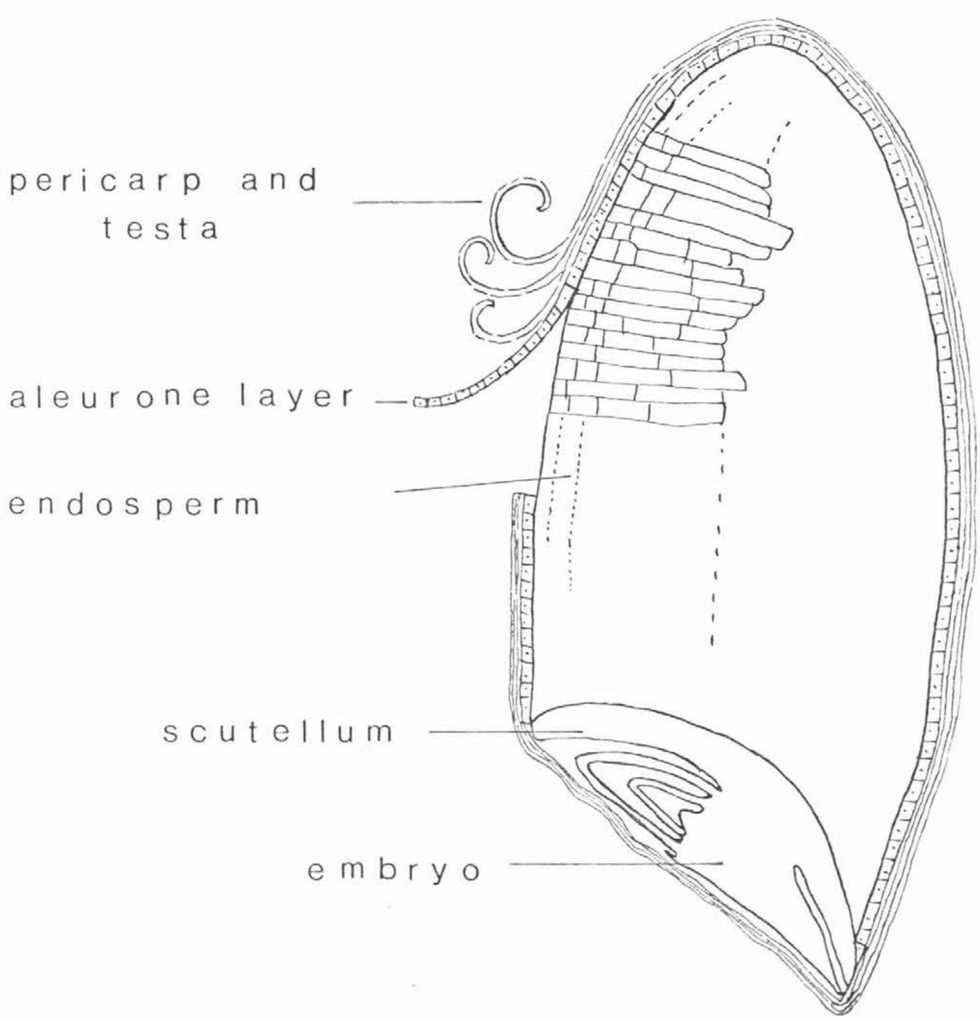
The group name originally given to enzymes catalyzing starch hydrolysis was diastase, later to be replaced by amylases. Amylases hydrolyze the α - 1, 4 - glycosidic linkage, most commonly being α or β amylase. α - Amylases are widely distributed in nature and split the bond in the interior of the substrate. 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 ($1 \rightarrow 6$) 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°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.

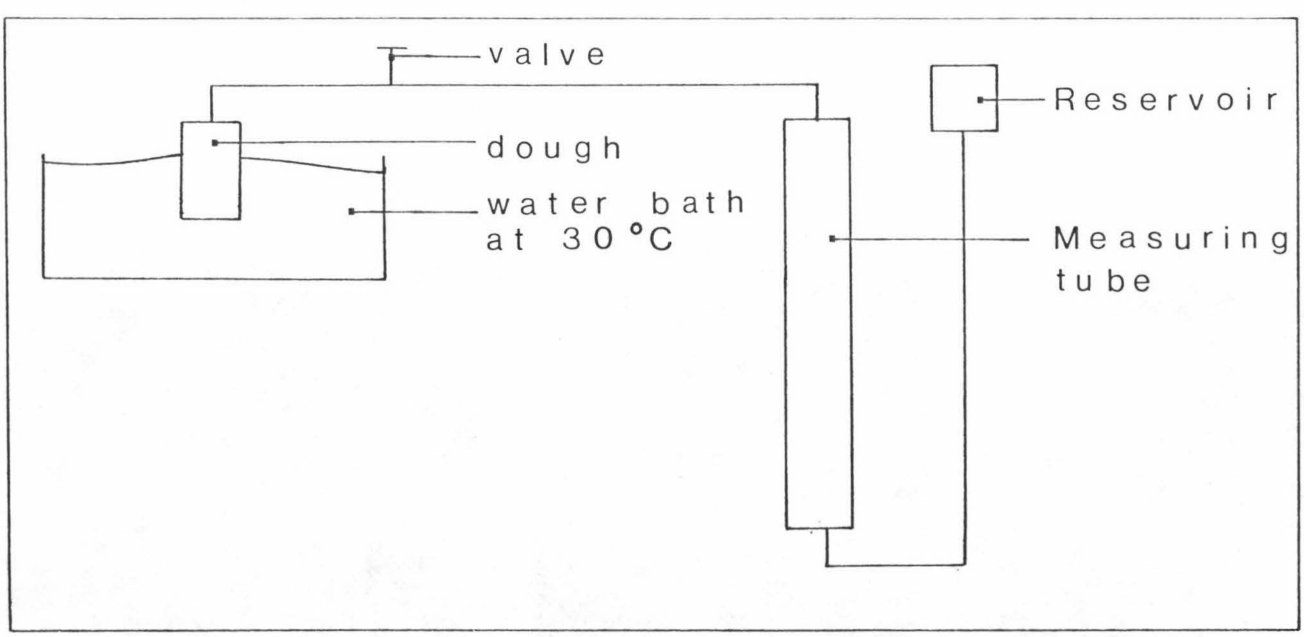
Measurement of gas production from a dough:

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 $10^{o}C$. 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 farino-graph, 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^{o}C. (Reed and Peppler, 1973). Inactivation of yeast occurs between 54-56^{o}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)

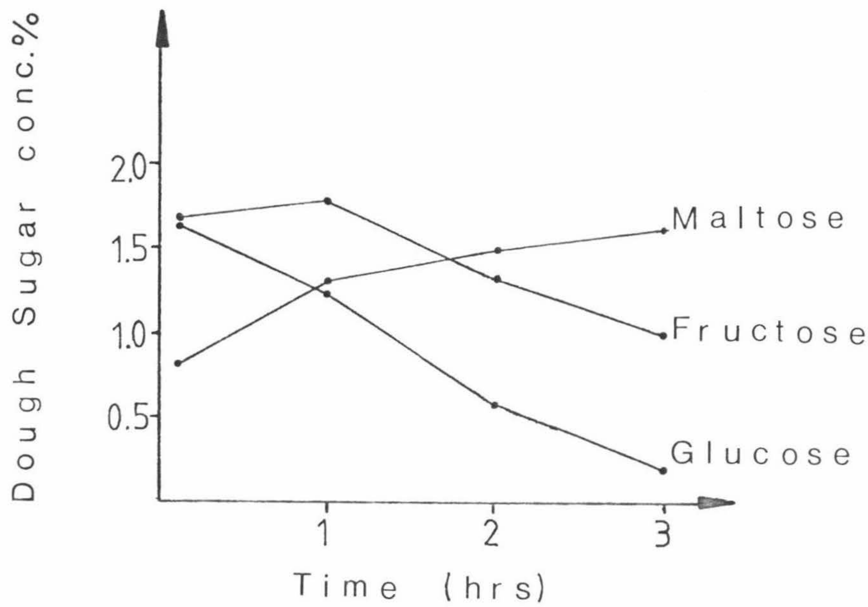
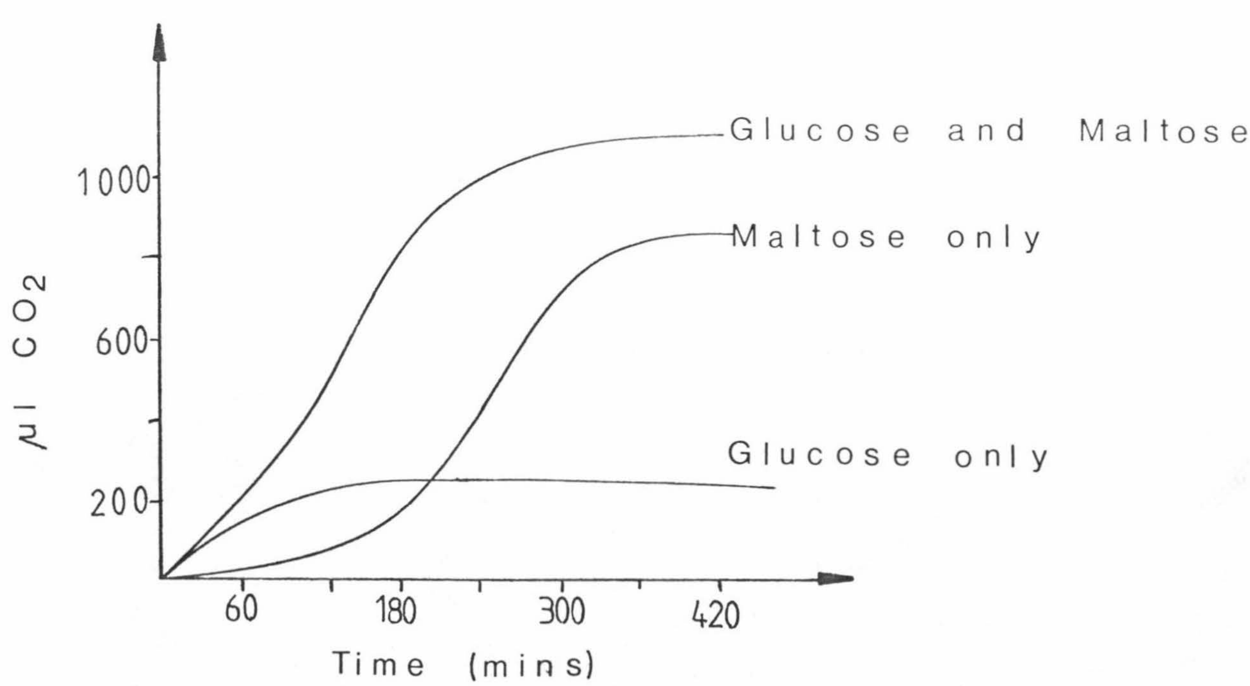


Fig. 1.6 Glucose and Maltose Fermentation by Baker's Yeast (Shieh *et al.*, 1973)



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^{o}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

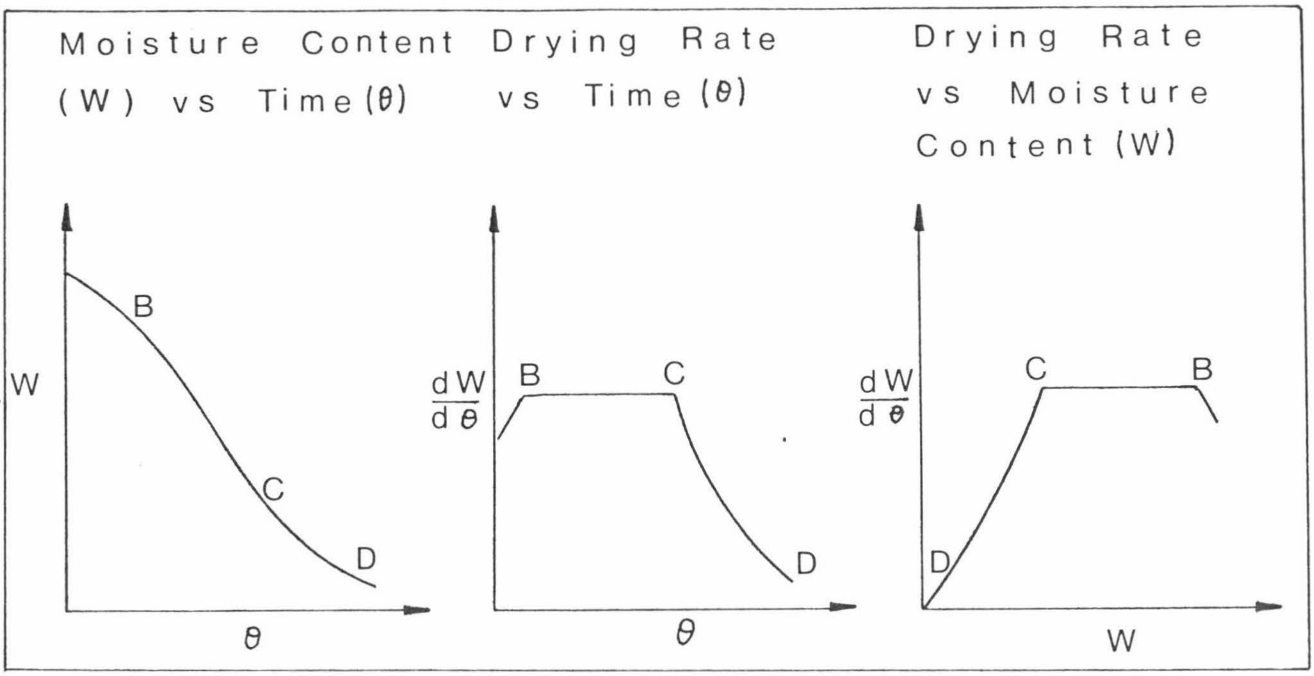
Removal of water during drying involves two mechanisms of heat and mass transfer. Firstly, when moisture is lying freely on the surface of the material being dried, the rate of removal is governed by the rate at which heat can be applied. Moisture removal is taking place at a constant rate. Secondly, 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: ratio ADY/solution	solution	time min.	temp. ^{o}C.	Author	Research Topic
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^{o}C.	Ponte _et al_. 1960	Studies on ADY
1/4	water	10	40-45^{o}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), hc= 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^{o}C. and 43^{o}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^{o}, 37^{o}, 32^{o}, and 28^{o}C. Generally, the air temperature does not exceed 45^{o}C. for a yeast bed temperature of 40^{o}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^{o}C. "Elevating strength" declined below 20% and increased slightly between 40 to 50% Hr. These authors concluded that temperatures between 30-50^{o}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°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 pòlyethylene and an outer layer of Aluminium foil, which is then heat sealed (Burrows, 1970).

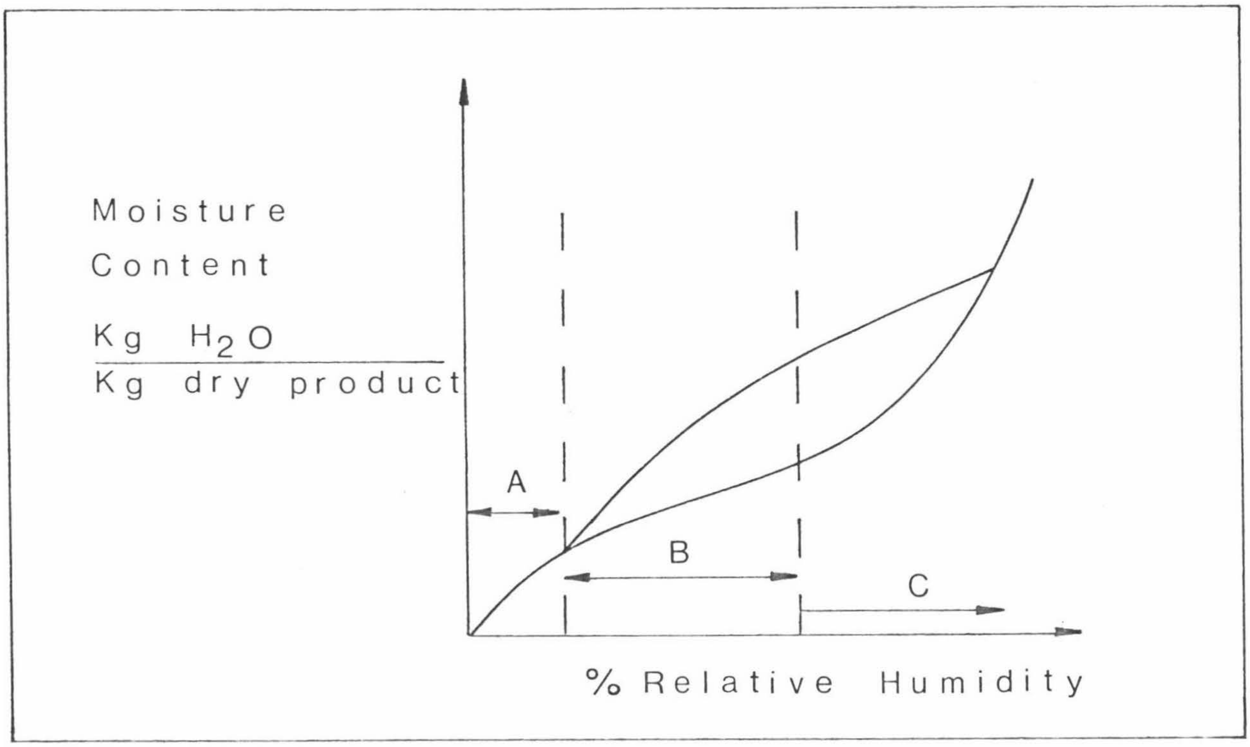
1.2.4 Isotherm Studies

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. 1.8 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°C. (White and Munn, 1951), though the commercial range is restricted to 25-35°C. The temperature for the optimal growth rate of yeast (35°C.) and CO_2 evolution (37°C.) differed from that for cell yield (30°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°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.

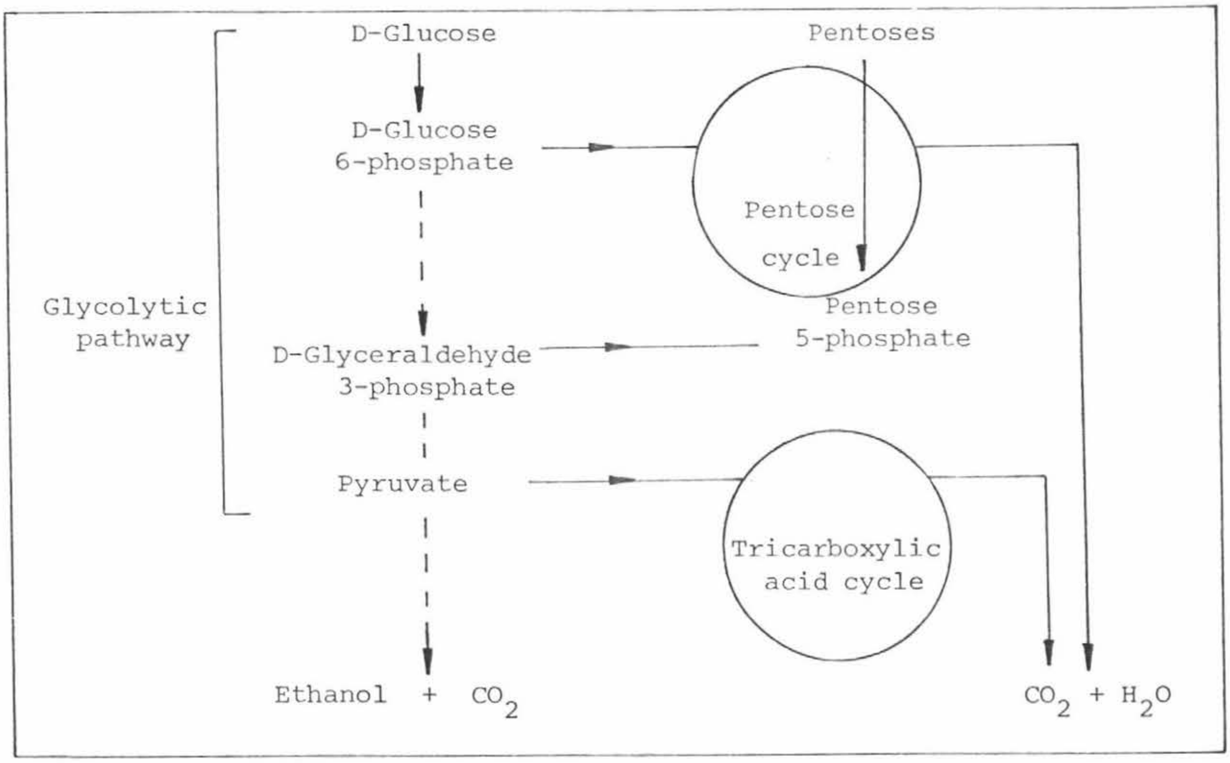
Sugar Uptake

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. Differences 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:

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

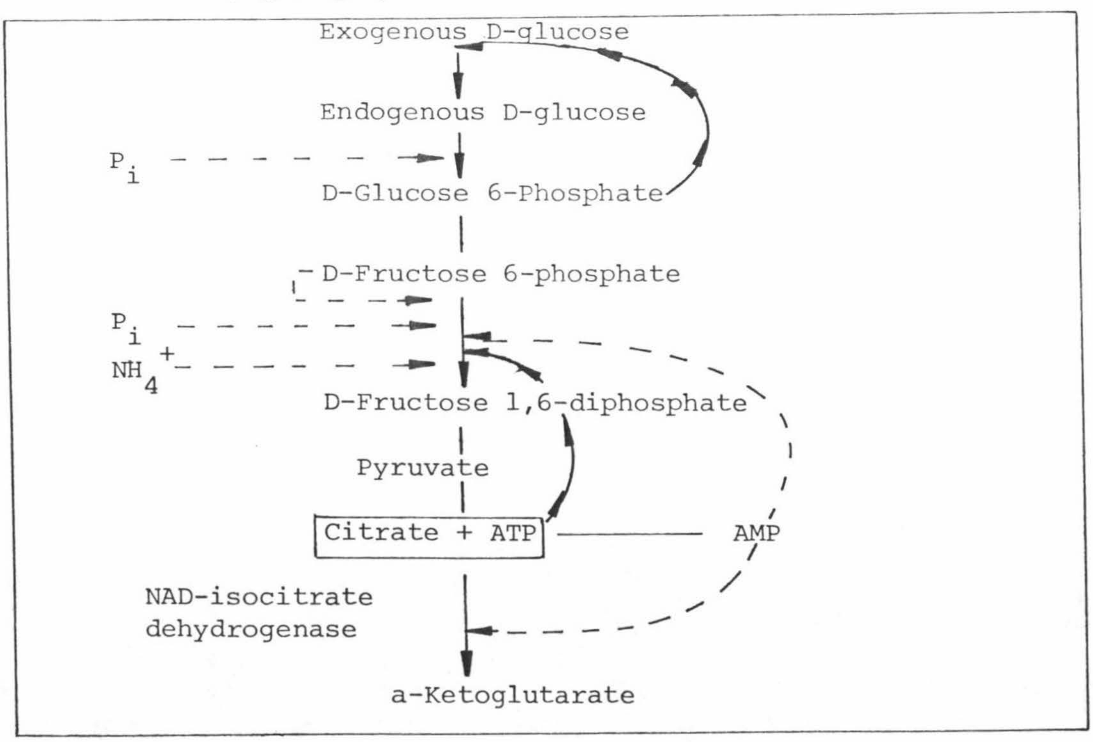
is dependent on allosteric activation by AMP (Hathaway and Atkinson, 1963). When the AMP/ATP ratio is low, the citrate concentration increases (see Fig. 1.10).

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.

2. METHODS AND MATERIALS

2.1 Yeast Assay and Storage Techniques

2.1.1 Yeast Cultures

In this section, the three strains of Saccharomyces cerevisiae used as inocula, are described.

Culture ATCC 7752 was obtained as a freeze-dried culture from the American Type Culture Collection (ATCC) Rockville, Maryland, U.S.A. This was reconstituted in M.R.S. broth and maintained on M.R.S. agar slopes (de Man et al., 1960). This particular culture, ATCC 7752, has been described as typifying the strain used to produce ADY (Reed and Peppler, 1973).

Culture F.I.1 was purified from fresh Compressed Baker's Yeast obtained from Fermentation Industries, N.Z. Ltd., Palmerston North. This was isolated and maintained on Yeast Morphology (Y.M.) agar (Difco Laboratories, Detroit, Michigan, U.S.A.).

Fresh Compressed Yeast was also used as an inoculum; one kg. blocks were obtained from Fermentation Industries, N.Z. Ltd., and stored at 4°C.

2.1.2 Storage of Yeast

The two pure cultures, ATCC 7752 and F.I.1 were stored on their respective maintenance agar slopes, M.R.S. and Y.M., at 4°C. and subcultured monthly.

Storage trials on Compressed Yeast were carried out in temperature controlled rooms at 20°C. and 4°C. The Compressed Yeast blocks, 0.1m x 0.03m x 0.02m were either stored loosely wrapped in waxed paper or polythene film, wrapped twice and sealed.

2.1.3 Measurement of Yeast Activity

The variety of methods available, including Warburg respirometric and dough leavening systems have been mentioned previously. In selecting a measuring device three criteria were used. Availability and cost of assembling the device, the relevance of the generated data and the accuracy and reproduc ibility of the data. It was considered that the Burrows and Harrison (1959) fermentometer formed the basis of a suitable yeast activity measuring device for the following reasons.

Gas production measurement was the technique chosen, thus eliminating the uncertainties of procedure and dough formulae as present in bake tests. Activity was expressed as millimole CO_2 / hr. / g yeast solid (Total solid). With wheat flour as the substrate and the yeast suspended in a salt solution (defined below) a dough closely related to a Baker's dough was produced. Using standards prepared for both commercial Compressed and Active Dry Yeasts, direct comparisons between samples were possible. Of the two gas production systems available, viz. constant volume or constant pressure, the latter system proved the simpler to construct and maintain. Increased accuracy was achieved by correcting between runs for temperature and pressure.

Modification to the Gassing Apparatus.

Two improvements were made to the basic design of Burrows and Harrison (1959). Firstly, a criticism of gas measuring systems has been the variation between apparatus. By using standard Quickfit (Q.F.) glassware this variation was reduced. Secondly, the original apparatus (Burrows and Harrison, 1959) consumed large amounts of materials, e.g. 4.0 g Compressed Yeast. By scaling down the apparatus, activity assays can be conducted on as little as 1.0 g Compressed Yeast and 0.3 g ADY without loss in accuracy.

Description of the Gassing Apparatus

The gassing apparatus consisted of two sections, firstly the test-tube (Q.F. M.F. 2412) with a cone/flexible tubing adaptor, (Q.F. M.F. 102) which was immersed in a water bath at 30°C. The water bath lid was built up with polystyrene foam insulation and when closed, with the circulation pump operating, the temperature was maintained constant at 30 $\pm$ 0.2°C. Secondly, using 5 mm I.D. rubber tubing the evolved gas passes through a Pyrex glass 3-way valve to a 25 ml gas measuring burette. Here the gas displaces a solution of calcium chloride (2.7% $^{w}/v$) and copper chloride (o.4% $^{w}/v$) into a conical socket (Q.F. SRB 19) which acts as a reservoir. The reservoir, on a movable stand can be positioned along the length of the burette. Two views of the apparatus are illustrated in Fig. 2.1 and a schematic diagram is shown in Fig. 1.4.

Operating Procedures

The yeast sample to be tested was suspended in a salt solution, (2.7 g NaCl and 0.4 g $(NH_4)_2SO_4$ per 100 mls) and made up to 25 mls. See paragraph entitled 'Calculation of sample size' (given below) for qualification of yeast sample size.

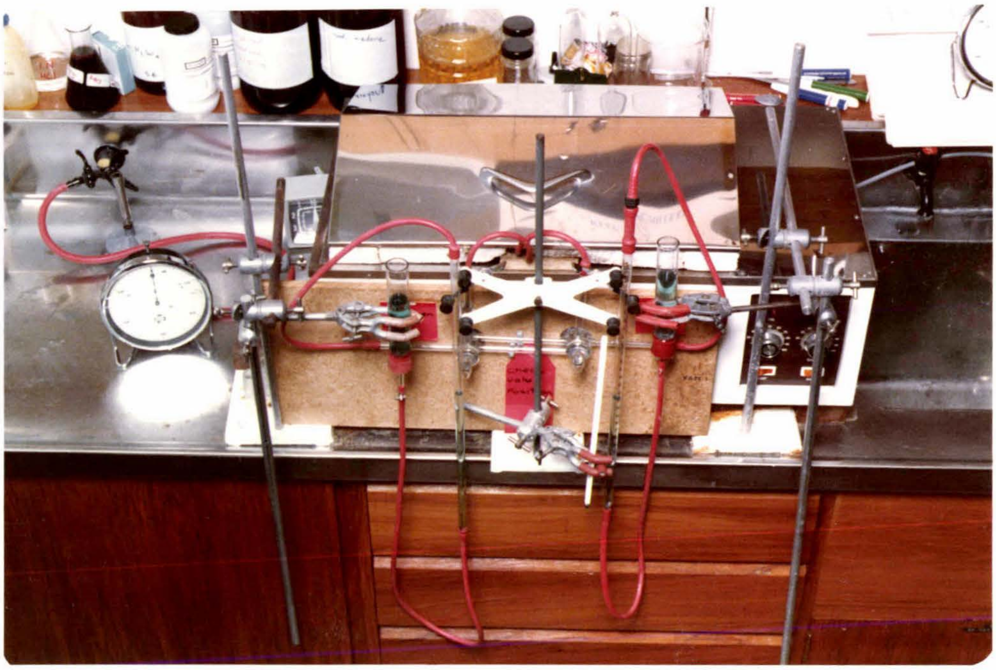
Step 1. By adjustment of the reservoir the manometer was brought to zero (atmospheric pressure) and the valve adjusted to isolate and seal off the connected Q.F. test-tube.

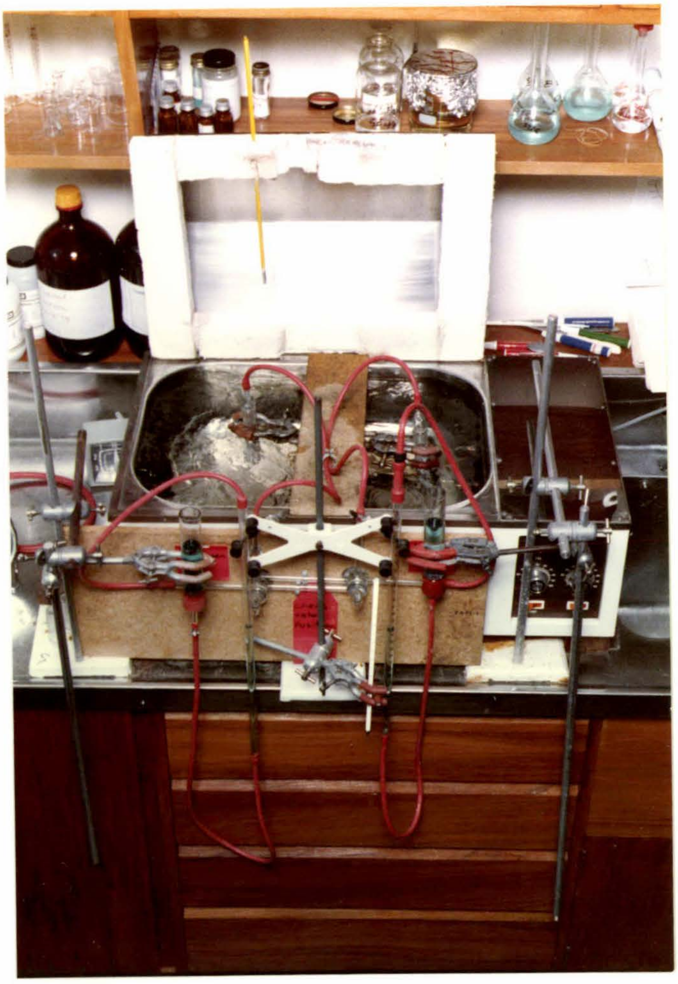
Step 2. 4 mls of sample yeast suspension were added to 5.0 g. of flour which had been incubated overnight at 30°C. in a stoppered test-tube. A timer was started and the flour/yeast solution mixed vigorously with a spatula for 30-40 sec. Any dough on the spatula was removed with a piece of moistened filter paper which was then added to the dough. The test-tube was then attached to the adaptor exactly 1 min. after mixing had commenced and immersed in the 30°C. water bath.

Step 3. Exactly thirteen minutes after first mixing, the gas volume in the test-tube and burette was connected by adjusting the valve. The timer was reset and the first reading of evolved gas taken 45 mins. later.

Step 4. By lowering the reservoir to the same level as the burette the pressure in the system was brought to atmospheric and the burette was read at precisely 45 min. The valve was closed, isolating the test-tube again and both the timer and manometer were reset to zero. The valve was re-opened and after a further 45 mins. a second reading

Fig. 2.1 The Yeast Activity Apparatus (see also section 1.1.3 for schematic diagram).





was taken. During the yeast activity assay, the room temperature and atmospheric pressures were recorded.

Calculation of Sample Size and Yeast Activity

The activity assay was based on the yeast total solid content. It was important to note that the amount of sample tested varied with the yeast's moisture content. An approximation was made initially, and the volume of gas produced was corrected according to the precise weight of total solids in the yeast suspension. An arbitrary standard was fixed corresponding to the amount of yeast in the dough. The dough consistency used by Burrows and Harrison (1959) and the modified apparatus is shown in Table 2.1. The total solid content of the yeast suspending solution corresponded to 3.78% and was used as a basal level. Calculations for converting observed mls. of gas evolved to mM CO_2 / hr. / g yeast solid are presented in Appendix 1.

Table 2.1 Experimental Dough Composition

	Dough Composition	
Components	I	II
Compressed Yeast (@ 31% Total Solids)	0.536 g	0.144 g
Suspending Solution	15.0 mls	4.0 mls
Flour	20.0 g	5.0 g

I Dough composition as listed by Burrows and Harrison (1959)

II Dough composition used in yeast activity assays.

Endogenous Respiration

The contribution of endogenous respiration to the overall yeast activity was examined. By replacing the flour substrate with an equal volume of washed sterile stand, any gas evolved from the mixture would be an indication of endogenous respiration. The activity assay was standard, as described above, in all other aspects.

2.1.4 Warburg Manometric Measurement

Studies of gas production by Compressed Yeast in a dough leavening system, using the yeast activity apparatus of section 2.1.3, were compared with similar studies using soluble substrates.

Fermentation of soluble substrates was by the direct Warburg method. Each Warburg flask (B. Braun, Melsungen, Federal Republic of Germany) contained 3 mls of nutrient solution at the concentrations listed in Table 2.2 (Shieh et al., 1973). Flasks were either flushed with dry nitrogen (food grade) for 10 min. to obtain anaerobiosis or air flushed. Gas evolved was expressed as $\mu l\,CO_2$ at S.T.P. i.e. $0^{\circ}C.$ and 760 mm Hg.

Table 2.2 Nutrient Solution for Warburg Manometer

Component		Concentration
Yeast Extract		5.0 mg
Yeast Suspension	(1)	1.0 mg
KH_2PO_4		4.556 mg
Sugar	(2)	Variable
Total volume		3 mls

(1) Compressed Yeast at 31% total solids

(2) Three substrates tested were glucose (5 mg), sucrose (2.5 mg), and maltose (2.5 mg) per reaction aliquot of 3 mls.

2.1.5 Rehydration and Foam Production

Active Dry Yeast must be rehydrated before an activity assay. As mentioned in section 1.1.5 and on the basis of section 3.1.6 the following technique was formulated.

Active Dry Yeast was rehydrated at $40^{\circ}C.$ in 2.5 mls of 2% $^{w}/v$ sucrose for 15 min. The resulting yeast slurry was made up to 25 mls with suspending (salt) solution and was assayed for yeast activity as described in section 2.1.3.

It was noted that those ADY samples producing no foam on rehydration also produced no gas during a later activity assay. Thus, incorporation of a foaming test into rehydration allowed a simple method for screening ADY. Samples giving little foam production were discarded while vigorously foaming samples were carried through to a full activity assay. This simple observation provided a rapid and simple screening test.

Generation of foam was estimated by rehydrating the sample in a

10 ml. measuring cylinder. Any sample whose volume exceeded 5 mls after 15 min. (i.e. 2 mls foam and 3 mls of ADY in sucrose solution) was assayed.

2.1.6 Other Analytical Procedures

(Moisture; dry weight; pH measurement; and residual sugar)

Instant Moisture Determination:

A Sauter Infrared Toppan balance (Model MPR 160/100 operating at 180 volts dried 1 g samples to constant dry weight without browning in 10-15 mins. Instant moisture determinations correlated with dry weight methods within ± 1% to levels of 10% moisture in the final product. Below this level, accuracy decreased and conventional dry weight methods were more appropriate. A drying curve for extruded yeast strands is shown in Fig. A1.1.

Dry Weight Determinations

Compressed and Dried Yeast: One gram samples, in duplicate, were weighed into pre-dried and tared moisture dishes and dried to constant weight at 105°C. overnight. All moisture determinations were expressed on a dry weight basis.

Total Solids: Five mls of sample were weighed into moisture dishes and dried to constant weight.

Fermentation Samples: Yeast cells from a 10 ml. sample were recovered, washed once in 10 mls of distilled water, and dried to constant weight as described above for total solids.

pH Measurement

A Metrohm instrument, Model E 350 B (Herisau, Switzerland) was used for all pH measurements.

Residual Sugar

Supernatant fluid from harvested yeast suspensions were analyzed for residual sugar. A Yellow Springs Instrument (YSI) Industrial Analyzer (Model 27) with a dextrose membrane (YSI # 2717 dextrose kit) was used for all determinations of D-glucose (YSI, Oct. 1978). Concentrations were expressed as mg/ℓ. Reproducibility is specified as ± 2% which from zero to the calibration point, is ± 4 mg/ℓ.

2.2 Drying

2.2.1 Compressed Yeast Preparation

Fresh commercial Compressed Yeast was obtained from a local producer in 1 kg blocks (Fermentation Industries, N.Z. Ltd., Palmerston North). This was stored at 4^{o}C. until used and discarded after 7 days. Freshly manufactured commercial Compressed Yeast exhibited the highest measured activity. As the drying experiments were likely to reduce this activity considerably, it seemed appropriate to commence an experiment with the highest possible initial activity.

The Compressed Yeast was suspended in distilled water containing an antioxidant mixture (described in Table 2.3) and an emulsifying agent (2 g of glyceryl monostearate in 5 mls corn oil). This gave a yeast slurry, of the composition listed in Table 2.3, which was extruded as 3.5 mm x 270 mm strands onto wire mesh drying trays.

After drying, all yeast samples were flushed with dry nitrogen and stored in air tight McCartney bottles at 4^{o}C.

Table 2.3 Composition of Yeast Slurry for Extrusion and Drying

Component	per 100 g
Yeast solids	24.87 g
Antioxidant (1)	0.13 ml
Emulsifier (2)	0.60 g
Corn Oil	1.40 ml
Total Solids	27.00 g

(1) Antioxidant mixture AMIF - 72 (Stecher, 1968) composed by weight of: 20% Butylated hydroxyanisole
6% Propyl gallate
4% Citric acid
70% Propylene glycol

(2) Glyceryl monosterate (Jones and Flowerdew, 1976).

2.2.2 Tunnel Tray Drying

The drying operation was carried out in a 'Climate Room' at the Department of Scientific and Industrial Research, Plant Physiology Division, Palmerston North. This allowed precisely controlled environmental conditions of humidity and temperature. Within the 2.75 m^2 room a tray drying tunnel was constructed, 1.3 x 0.2 x 0.2 m, with a cross sectional area of 0.04 m^2.

Air from the room was blown through the tunnel by a small electric fan. Four sets of air-flow spoilers, of decreasing box size directed the air over two stainless steel trays (2 mm mesh), fitted 0.05 m and 0.1 m from the tunnel floor. The average airflow through the tunnel was determined at several points and measured with a thermo-anemometer (Wallac-Oy Finland). Temperature measurements were made using 0.01 mm copper-constantan thermocouple wires in conjunction with an Esterline Angus recorder. The recording system was set against an ice junction. The thermocouple wires were located in the yeast strand, the inlet air stream, and outlet air stream.

A schematic diagram of the apparatus is shown in Fig. 2.2. The conditions under which the tunnel operated are presented in Table 2.4.

Table 2.4 Tunnel Tray Drying Conditions

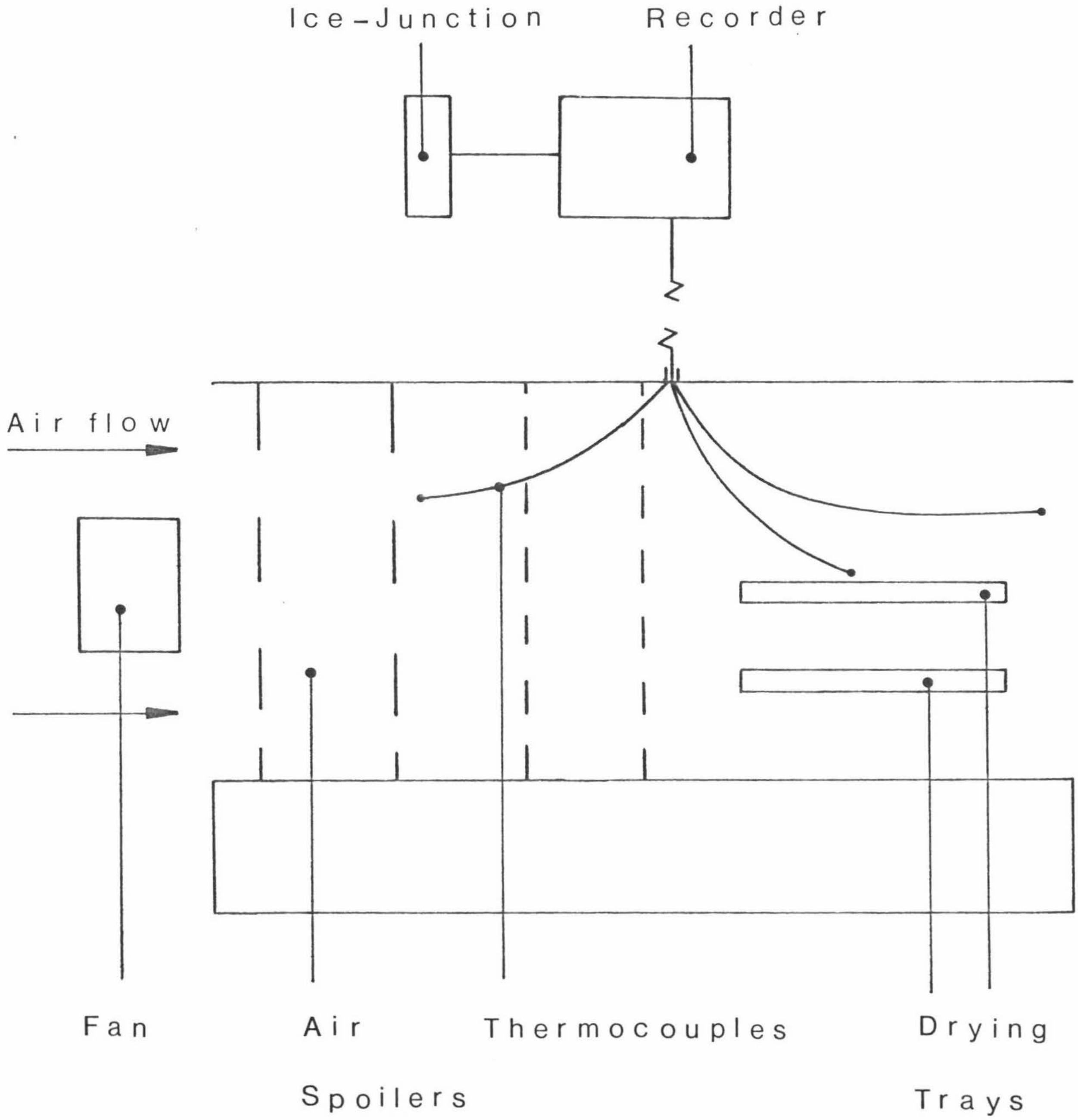
Drying Parameter	Operating Value
Constant Conditions	
Air temperature	$40 \pm 0.25^{o}C.$
Air speed	1.5 ± 0.1 m/s
Final moisture content (dry weight)	$9 \pm 0.25\%$ ($^{w}/w$)
Variable Conditions	
Air humidity (Hr% @ $40^{o}C.$)	$45 \pm 1\%$
	$31 \pm 1\%$
	$17 \pm 1\%$
Drying additives (1)	present
	absent

(1) The additive mixture was composed of an antioxidant (0.13% AMIF - 72) and 2% glyceryl monostearate.

2.2.3 Cabinet Tray Drying

The optimum conditions for drying were scaled down from a tray drying tunnel to a laboratory cabinet. This allowed small amounts of Compressed Yeast to be examined without the expense of operating the larger tunnel as described in section 2.2.2.

Fig. 2.2 Schematic Diagram of the Tray Drying Tunnel



A standard laboratory incubator was used with a drying tray suspended through the top ventilator (see Fig. 2.3). By connecting the tray to a triple-beam balance the weight loss of water could be monitored without destruction of the sample. The stainless steel tray (1.5 mm mesh and 11.5 cm dia.) was suspended in a humidity chamber above a magnetic stirrer (Cemco Instruments, Breda, The Netherlands).

Inside the chamber, a saturated solution of magnesium chloride (250 mls) gave a humidity of 31% Hr at 40^{o}C. (Rockland, 1960). A stand in the chamber supported the central shaft, upon which a magnet stirred the saturated solution. It also turned an acrylic plastic fan of the same diameter as the chamber and tray. This was arranged immediately above the $MgCl_2$ solution.

This circulated air around the drying tray at 1.5 m/sec. Air inside the cabinet was also held at 31% Hr using saturated solutions and monitored with wet and dry bulb thermometers. An electric fan circulated air over the thermometers and around the cabinet.

Weight loss was recorded every 15 mins. with the temperature and humidity adjusted if required. The weight recorded was not influenced by air movement as the fans were stopped for each measurement. Temperature measurements were made using 0.5 mm copper-constantan thermocouple wire and recorded with a Honeywell Versa Print (Model FR 801). Thermocouple wires were located in the cabinet as well as above the drying tray in the humidity chamber. Drying data was analyzed in accordance with accepted drying theory (Perry, 1963).

The apparatus is illustrated in Fig. 2.4, together with a schematic diagram, Fig. 2.3. Table 2.5 summarises the operating conditions used during cabinet tray drying.

Fig.2.3 Schematic Diagram of the Humidity Chamber

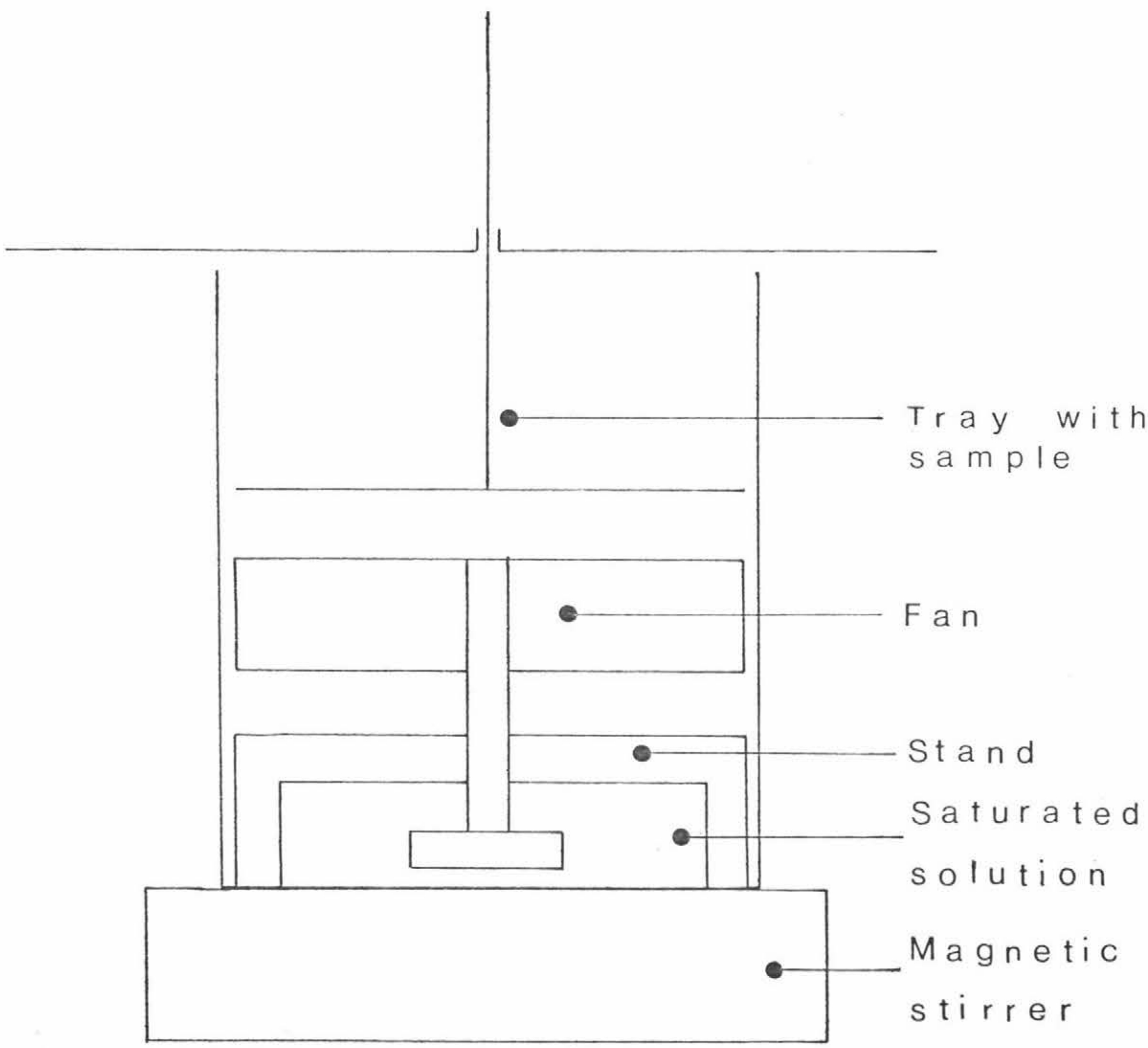


Fig. 2.4. Cabinet Tray Drier

Table 2.5 Cabinet Tray Drying Conditions

Drying Parameter	Operating Value
Constant Conditions	
Air Speed	1.5 ± 0.1 m/s
Final moisture content (dry weight)	$9 \pm 0.25\%$
Antioxidant	0.13 ± 0.005 %
Relative humidity @ 40°C.	$31.0 \pm 1.5\%$
@ 35°C.	$32.0 \pm 1.5\%$
Variable Conditions	
Air temperature	$40.0 \pm 0.5^{\circ}$C.
	35.0 ± 0.5
Glyceryl monostearate	present
(2%)	absent

2.2.4 Other Drying Methods Investigated

Acetone Drying

The initial temperature of acetone and Compressed Yeast was varied from -20°C. to 20°C. to produce Active Dry Yeast without heat treatment. Fifteen grams of commercial Compressed Yeast were crumbled into 250 mls of acetone and stirred for 2 mins. This slurry was filtered for 2 mins. through a Hartley funnel using Whatman No. 40 ashless filter paper. The filtered yeast bed was allowed to air dry for 1 hr. at 20°C. Drum grade acetone, distilled to B.P. 56.5 - 57.5, was used for extraction. No additives were mixed with the yeast.

Freeze-Drying

Ten grams of commercial Compressed Yeast were crumbled into a 100 ml round bottom flask (QFFR 100/2S). No additives were included and the flasks were frozen at -20°C. The rate the yeast was frozen at was determined using thermocouples inserted in an equivalent quantity of Compressed Yeast. A Virtis bench top freeze-dryer (Model 10-020) was used and connected to a Cemco Hyvac 7 (Model 91506 - 1) vacuum pump. After lyophilization, over a 10 hr. period, the vacuum system was returned to atmospheric pressure and the samples flushed with dry nitrogen.

2.2.5 Isotherm Studies

The methods available for the determination of sorption isotherms were reviewed by Gål (1975). The discontinuous registration of weight changes was used for isotherm determination.

Eight Relative Humidity Cabinets containing the saturated solutions, as listed in Table 2.6, were used.

5 g samples of commercial Active Dry Yeast were spread on petri dishes and allowed to equilibrate for 7 days at 20°C. Using 1 g samples the moisture content was determined. Samples which lost water were moved to the next highest humidity, those which gained moisture were moved to the next lower humidity value. This effectively examined the samples from both the rehydration and dehydration mode. From the weight data, an isotherm was established by plotting Dry Weight versus Relative Humidity.

Table 2.6 Relative Humidities of Saturated Solutions at 20°C. (Rockland, 1960).

Saturated Solution	% Relative Humidity
Lithium chloride	12
Potassium acetate	23
Magnesium chloride	33
Magnesium nitrate	52
Sodium bromide	57
Cupric chloride	68
Sodium chloride	75
Potassium chloride	86

2.3 Fermentation

2.3.1 Growth Media

Basal Medium

A semi-synthetic basal medium was used for growth and activity trials. This medium removed the uncertainties associated with molasses composition. Medium composition is shown in Table 2.7.

This medium is based on that of Olson and Johnson (1949) with yeast extract replacing the original amino acids. The yeast extract was supplied by E. Merk Ltd., Darmstadt, Germany and all remaining ingredients (A.R. grade) by B D H Chemicals Ltd., Poole, England.

Table 2.7 Composition of Basal Medium

Component	Concentration per ℓ.
Glucose	10.0 g
$NH_4H_2PO_4$	6.0 g
KH_2PO_4	0.2 g
$MgSO_4 \cdot 7 H_2O$	0.25g
Yeast Extract	5.0 g
Ferrous Ammonium Sulphate	150.0 μg
Zinc Sulphate	400.0 μg
Copper Sulphate. 5 H_2O	25.0 μg

The pH was adjusted to 5.0 with dilute H_3PO_4.

Sterilization was at 121°C. for 15 min.

Alterations to the Basal Medium

When examining the effect of growth media composition on Compressed Yeast activity the glucose and nitrogen concentrations were varied, that is, the carbon to nitrogen ratio differed. The remaining media components were unchanged. Table 2.8 lists the glucose and nitrogen content of the media at the two concentrations studied.

Table 2.8 Carbon and Nitrogen Content of Media

Medium	Total Carbon (ppm)	Total Nitrogen (ppm)
Basal Medium	5300 (1)	1230 (2)
minus Glucose @ 1% ($^w/v$)	1695	
minus $NH_4H_2PO_4$ @ 0.6% ($^w/v$)		500
<u>High level</u>		
Glucose @ 3% ($^w/v$)	12510	
$NH_4H_2PO_4$ @ 1.2% ($^w/v$)		1960
<u>Low level</u>		
Glucose @ 0.5% ($^w/v$)	3495	
$NH_4H_2PO_4$ @ 0.3% ($^w/v$)		865

(1) Total Carbon analyzed by N.Z. Forest Products, Tokoroa using Oceanics Ltd., Total Carbon Analyzer.

(2) Includes yeast extract at 10% ($^w/v$) total nitrogen, as specified by E. Merk (1979).

Maltose Medium

An alternative to the basal medium was used when, after growth, culture ATCC 7752 gave low activity values. The medium's composition per ℓ was 13.0 g Nutrient Broth (Oxoid Ltd., London), 20.0 g yeast extract 10.0 g maltose; sterilization was at 121^{o}C. for 15 min.

Molasses Medium

Molasses was used, firstly, for a comparison between commercial and synthetic media and secondly, to replace glucose in the basal medium. Molasses was obtained in a diluted state (S.G. 1042) from Fermentation Industries Ltd., Palmerston North. This was then sterilized at 110^{o}C. for 10 min. aseptically centrifuged at R C F 5,000 x g to remove sludge and decanted into sterile flasks.

A Built Up Medium

To investigate the effect of growth media on dried yeast activity a sequential medium was developed. This involved a peptone base of 0.5% (^{w}/v) Peptone Water (Oxoid Ltd., London), to which was added the basal medium without glucose and finally a sugar source of either glucose at 3% (^{w}/v) and 0.5% (^{w}/v) or molasses.

2.3.2 Fermentation Procedures and Parameters

Fermentation Conditions

To increase the cell population a subculture stage was used between the maintenance storage slope and the actual fermentation run. This intermediate subculture of 10 mls, incubated at 30^{o}C. for 24 hrs., produced a standardized inoculation (10 mls/ℓ @ 3 mg dry weight/ml) for each fermentation run.

For each set of fermentation conditions, the contents of replicate shake flasks were pooled to a final volume of 1 ℓ, or 2 ℓ in cases of poor growth. Erlenmeyer flasks (500 mls) containing 250 mls of culture medium were incubated as per Table 2.9 on a Gallenkamp orbital shaker at 250 r.p.m., though an hour's rest was allowed before harvest (see section 1.3.2). Standing cultures were incubated again as per Table 2.9. Yeast cells were harvested by centrifugation at R C F 8,000 x g at 10^{o}C. for 15 min. The supernatant fluid was discarded and the cells resuspended in 50 mls. distilled water, then centrifuged at R C F 14,000 x g for 15 mins. at 10^{o}C. An MSE Hi-Spin Centrifuge (Scientific Instruments, Manor Royal, Sussex, England) was used for harvesting. The higher centrifuge speed used to recover the washed cells produced a slug of Compressed Yeast. This was similar in total solids to commercial Compressed Yeast. The yeast was stored at 4^{o}C. for no longer than 12 hrs. before drying experiments were commenced.

Fermentation Parameters

The parameters studied in this investigation of Compressed Yeast activity are shown in Table 2.9. The parameters chosen include media as well as environmental conditions.

To establish the functional relationship between variables, regression analysis or experimental design is often employed (Lipson and Sheth, 1973). This approach has been used to quantify physical engineering systems and more recently microbial growth response (Schroder and Weide, 1974; Eroshin et al., 1976).

Statistical analysis of data, including both drying and fermentation sections, was carried out using a statistical computer programme, MINITAB. A variety of statistical functions are possible, from correlation of data to multiple linear regression (Ryan, 1976).

Table 2.9 Experimental Fermentation Parameters

Fermentation Parameters	Experimental Design factor	Actual values high + 1	low - 1
Temperature	A	37^{o}C.	28^{o}C.
Dissolved oxygen	F	agitated	standing
Initial Medium			
pH	B	6	4
nitrogen	C	0.3%	1.2%
carbon	E	0.5%	3%

3 RESULTS

3.1 Measurement of Yeast Activity

3.1.1 Yeast Culture Screening and Strain Selection

For fermentation studies it was desirable to select a pure culture which, after growth in laboratory media, demonstrated activities equal to or better than that of commercial Compressed Yeast.

Two cultures were available for this purpose, S. cerevisiae ATCC 7752 and a laboratory isolate F.I. 1 originating from commercial Compressed Yeast. The cultures were grown in basal medium but because of the persistently low activity values of ATCC 7752, it was also tested in a maltose medium (see section 2.3.1). The controls chosen were freshly manufactured commercial Compressed Yeast and Active Dry Yeast. These were not grown in a laboratory medium but subjected directly to activity measurements. Laboratory grown preparations were assayed after cell harvest. Drying to the active dry form was not conducted.

Results of the screening of the laboratory grown yeast cultures are shown in Table 3.1. Repeated subculturing of ATCC 7752 on a maltose based medium did not increase its activity. This made it unsuitable for any studies where a direct comparison with commercial products was desired. It can be seen that F.I. 1 gave an activity which approached that of freshly prepared Compressed Yeast. For this reason F.I.1 was chosen for further studies, namely the effect of growth medium constituents on yeast activity.

Table 3.1 Screening of Yeast Culture Activity

Yeast Preparation	Laboratory Growth Medium	Activity (1)
Laboratory Cultures		
ATCC 7752	Basal	8.3
ATCC 7752	Maltose	8.2
F.I.1	Basal	14.4
Controls		
Commercial Compressed	Nil (2)	16.9
Active Dry	Nil (2)	9.9

(1) Activity expressed as mM CO_2/hr./g. yeast solid

(2) Commercial Compressed and Active Dry Yeasts were assayed directly from the package with no intermediate growth stage as in laboratory cultures.

3.1.2 Storage of Compressed Yeast

For the purpose of investigating the long term stability of Baker's Yeast activity, two storage temperatures ($20^{o}C$. and $4^{o}C$.) were selected as representing room temperature and refrigerated storage respectively. Two covering materials were used, loosely wrapped waxed paper and sealed polythene film. The activity of the compressed yeast after storage is shown in Table 3.2.

Activity decreased under all storage conditions except at $4^{o}C$. in waxed paper. The worst storage conditions was in sealed polythene film at $20^{o}C$. Possible explanations for the observed activity decline are firstly the occurrence of endogenous metabolism. The consumption of glycogen, fat, protein, and nucleic acids leading to eventual cell death could account for less than 5% of the original activity remaining after 21 days in sealed polythene film. Secondly the presence of toxic metabolic products would also contribute towards activity loss. Using a polythene film covering, it is expected that glycolysis was occurring with the subsequent accumulation of ethanol, organic acids, and CO_2. With a waxed paper covering these effects of fermentative metabolism would be reduced. Thirdly, it is possible that polymers toxic to yeast cells were leached from the polythene covering during storage. A disadvantage of waxed paper covering was the drying of the yeast cake. This resulted in a dark brown cake which was difficult to resuspend. It seemed possible that with both covering materials, storage at $4^{o}C$. slowed the rate of endogenous metabolism compared to storage at $20^{o}C$. and thus activity was maintained.

This trial clearly indicated that storage of wax paper wrapped Compressed Yeast at $4^{o}C$. was the better of the two procedures tested.

Table 3.2 Compressed Yeast Storage Trial

Storage Wrapping	Waxed paper		Polythene film	
Storage Temp.	$4^{o}C$.	$20^{o}C$.	$4^{o}C$.	$20^{o}C$.
7 days	16.8	17.4	16.6	14.1
14 days	17.0	16.7	15.8	3.0
21 days	17.7	6.4	12.6	0.7

All activity values expressed as mM CO_2/hr/g. yeast solid.
In all experiments, the initial activity was 17.2.

3.1.3 Endogenous Respiration

In considering yeast activity it seemed appropriate to recognise the contribution endogenous metabolism was making to the measured total activity. It was hypothesised that those yeast preparations with very low overall activity might in fact be exhibiting endogenous respiration. In Table 3.3 yeast preparations with high and low activity are compared with a control.

Where no substrate was available endogenous respiration would account for almost the entire gas produced. There was zero gas production in the second 45 min. "rising" period. Endogenous respiration accounted for $^2/3$ of the initial gas production of an active yeast with a flour substrate. An increase in the sucrose concentration used in rehydration did not give any corresponding increase in activity.

Low activity yeast cultures were, therefore, unable to use a flour or sucrose substrate and it appeared that the observed gas production was the result of endogenous respiration.

Table 3.3 Endogenous and Exogenous Metabolism in Yeast

Yeast	Substrate	Activity (1)	
		After 13 min.(2)	After 103 min.
Compressed	Nil (control)	1.1	1.1
Compressed	Flour	1.6	17.0
ADY (3)	Flour, with 2% ($^w/v$) sucrose at rehydration	0.9	1.0
	Flour, with 20% ($^w/v$) sucrose at rehydration	1.0	1.0

(1) mM CO_2/hr./g yeast solid

(2) Average gas production after 13 min.

(3) Active Dry Yeast prepared in the laboratory and typical of preparations of low overall activity.

3.1.4 A Comparison of Methods for Activity Measurement

This section considers yeast activity measurements in terms of sugar utilization. Monitoring was carried out by two methods, firstly the Warburg manometric technique, and secondly a dough leavening system.

Soluble Substrate Studies

Traditional manometric measurement of yeast fermentation activity is by the direct Warburg respirometric method. The fermentation results of such studies utilizing three sugars are shown in Fig. 3.1. Each Warburg flask (see Methods section) was initially flushed with pure dry nitrogen as conditions in a Baker's dough are likely to approach the anaerobic state. This did not shorten the fermentation time as shown by comparing results for flasks flushed with nitrogen and air at 2.5 mg sucrose. Many studies show gas production over extended time intervals, up to 6 hrs. In breadmaking, however, the proofing and resting stages do not normally continue beyond 100 mins. At this time gas production rate was constant as shown in Fig. 3.1.

By expressing the results of Fig. 3.1 in terms of yeast activity (millimoles CO_2/hr./g final yeast solids), which is essentially the rate of gas production, Fig. 3.2 was generated. It was found that the rate of gas production varied with the nature of the fermentation substrate. Illustrated in Fig. 3.2 is the data for those experiments which used 5.0 mg glucose, 2.5 mg sucrose and 2.5 mg maltose. After fermenting for 100 minutes, the rate of gas production was constant. The gas rate can be misleading in the early stages of fermentation. Endogenous respiration, which can produce up to two thirds of the initial gas production (section 3.1.3) was thought to contribute towards the high initial gas rate. It seemed appropriate therefore, to delete the first two data points in Fig. 3.2.

Dough Manometer Studies

The yeast activity manometer, as described in section 2.1.3, is

Fig.3.1 Warburg Fermentation of Sucrose, Glucose and Maltose

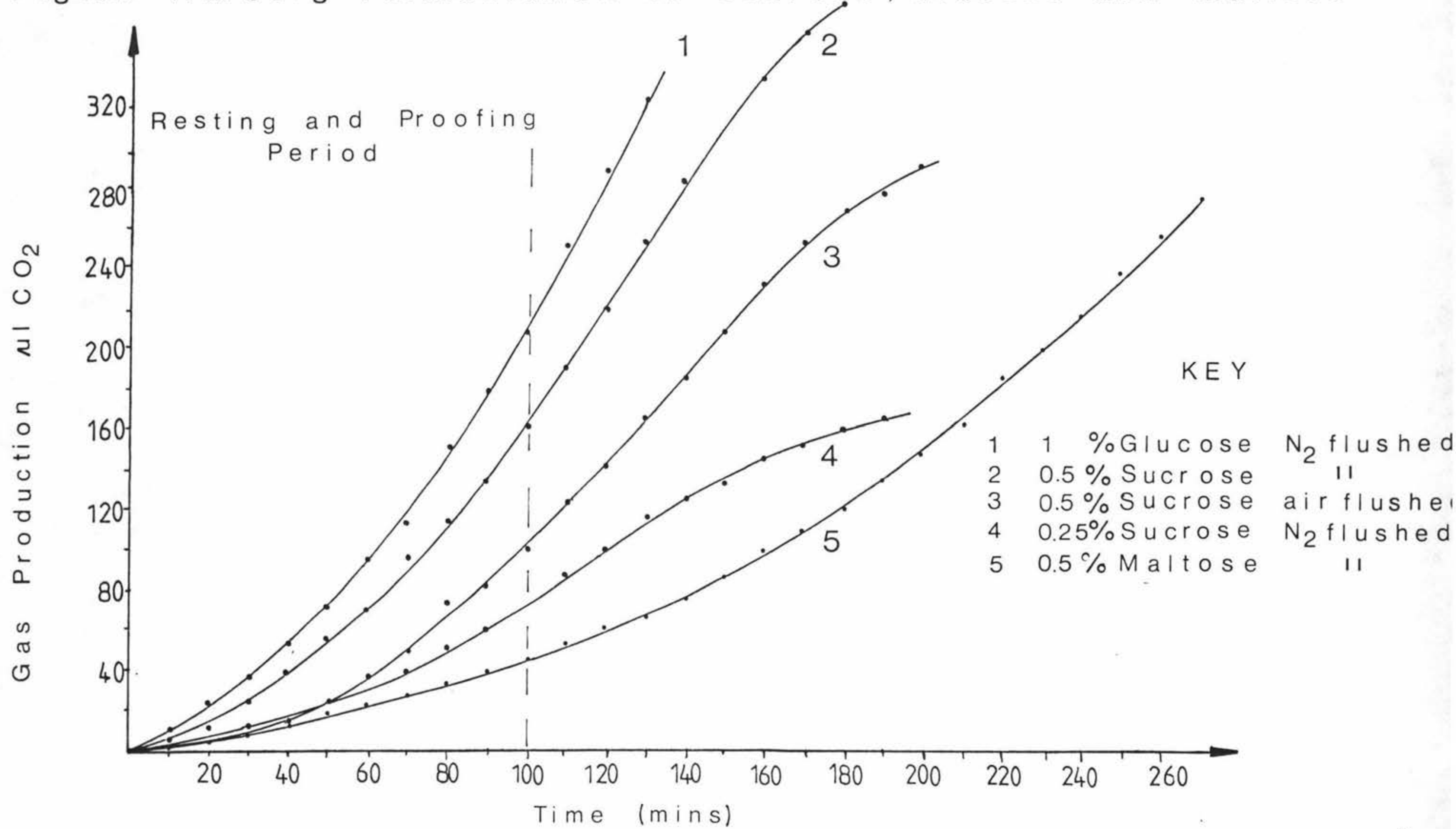
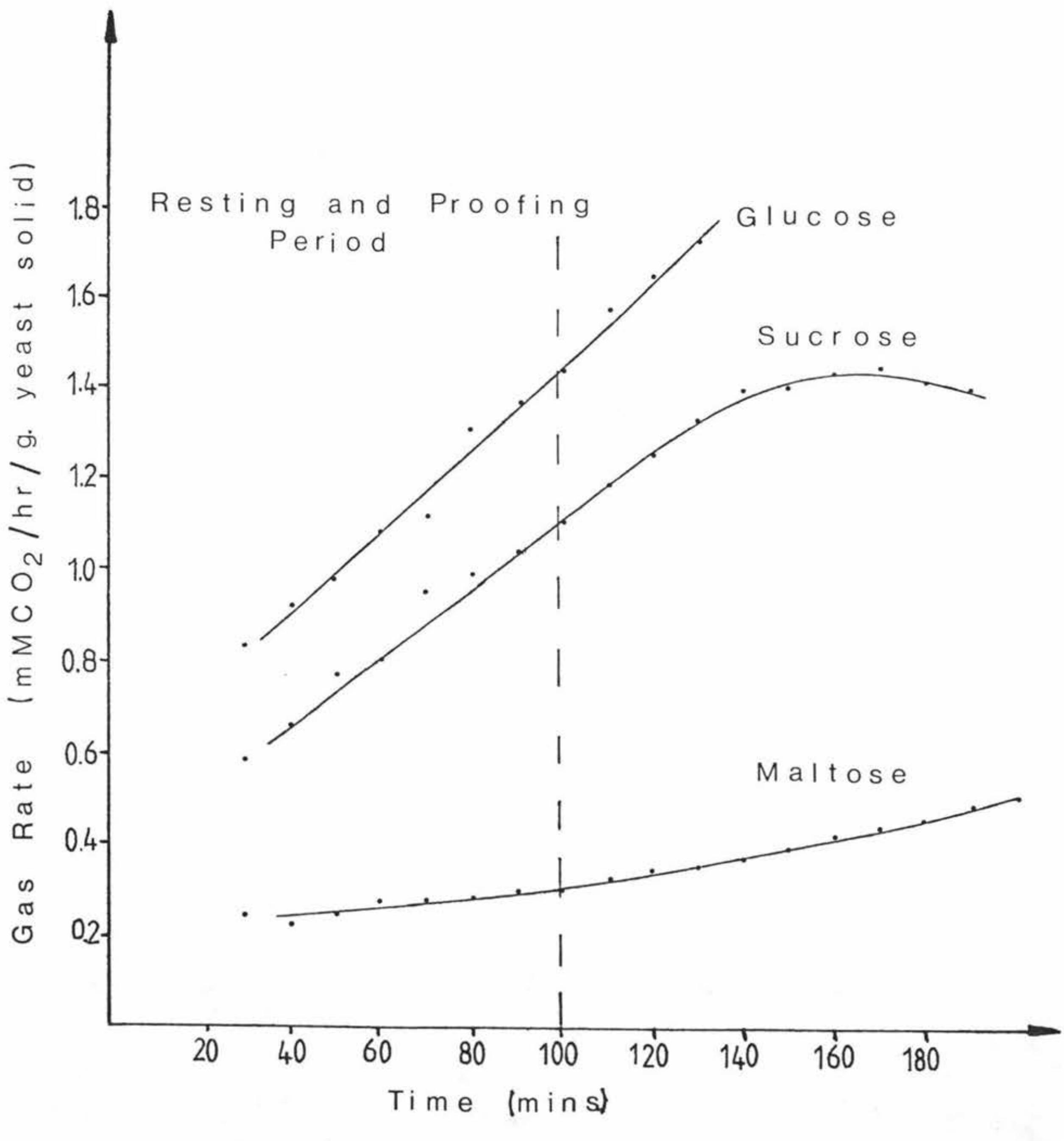


Fig. 3.2 Rate of Gas Production for the Warburg Manometric Method



an example of a dough leavening system. This apparatus can be used as an incremental measuring system, and the gas evolved from a flour substrate is shown in Fig. 3.3. Gas production was constant during the time interval under study, as in the Warburg studies, although a shorter lag period was evident. The rate of gas production (millimole CO_2/hr./g final yeast solids) was found to reach a maximum after 100 mins. fermentation, Fig. 3.4.

A direct comparison cannot be made between the two yeast activity methods due to the nature of the substrates. None of the three sugars, glucose, sucrose and maltose, at the tested substrate concentrations produced a rate curve similar to that obtained from flour. The shorter lag period with the flour substrate could arise because initially there is a mixture of natural sugars in flour and such combinations are known to induce enzymes e.g. glucose induction of maltose enzyme systems (Lovgren and Hautera, 1977). For a practical comparison between soluble substrates and dough systems a similar sugar utilization rate is required. The soluble substrate combination and concentration necessary to obtain these conditions must be determined.

3.1.5 Foaming Test

The volume of foam produced when a dried yeast sample was rehydrated appeared to be related to metabolic activity. From this observation it was hypothesised that foam production could be applicable as a rapid screening test for low activity preparations of yeast (see methods 2.1.5). Only those samples with an arbitrarily chosen foaming rate were subjected to the more detailed and accurate manometric procedure.

Commercial Compressed Yeast was dried in a cabinet tray drier and samples analyzed each hour for both metabolic activity and foaming ability. Foaming activity followed gas metabolic activity and both parameters appeared directly related to each other (Fig. 3.5). From these experimental data it was concluded that this connection could be used to screen dried yeast samples.

The manometric activity and foam production is compared in Table 3.4. It seemed appropriate to subject to further testing those samples of yeast which produced greater than 5 mls foam in 15 mins. This was the "cut off" foaming point. On some occasions the foaming of suspensions of Compressed Yeast was so vigorous that

Fig. 3.3 Fermentation of Flour using a Yeast Activity Manometer

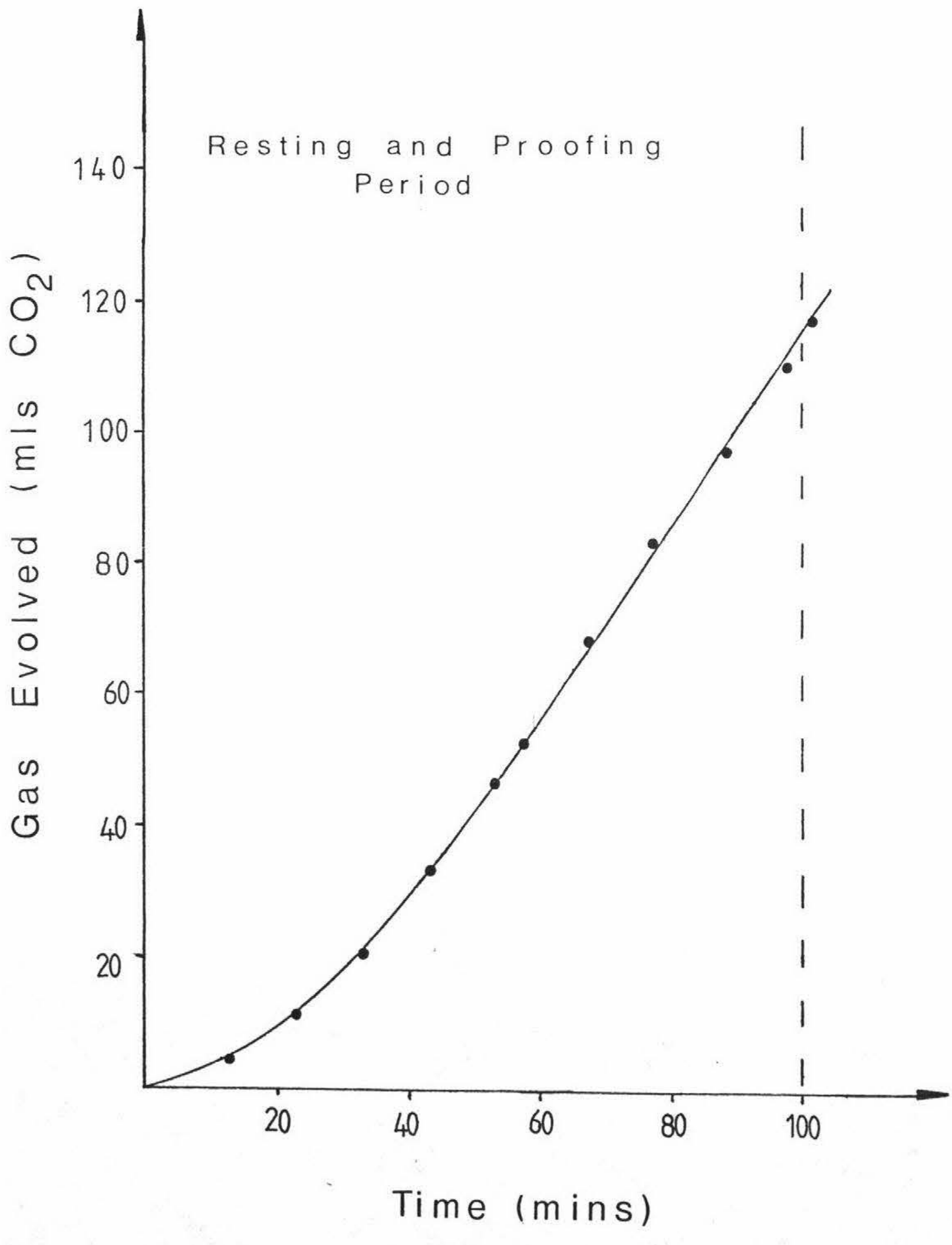


Fig.3.4 Rate of gas Production in the Yeast Activity Manometer

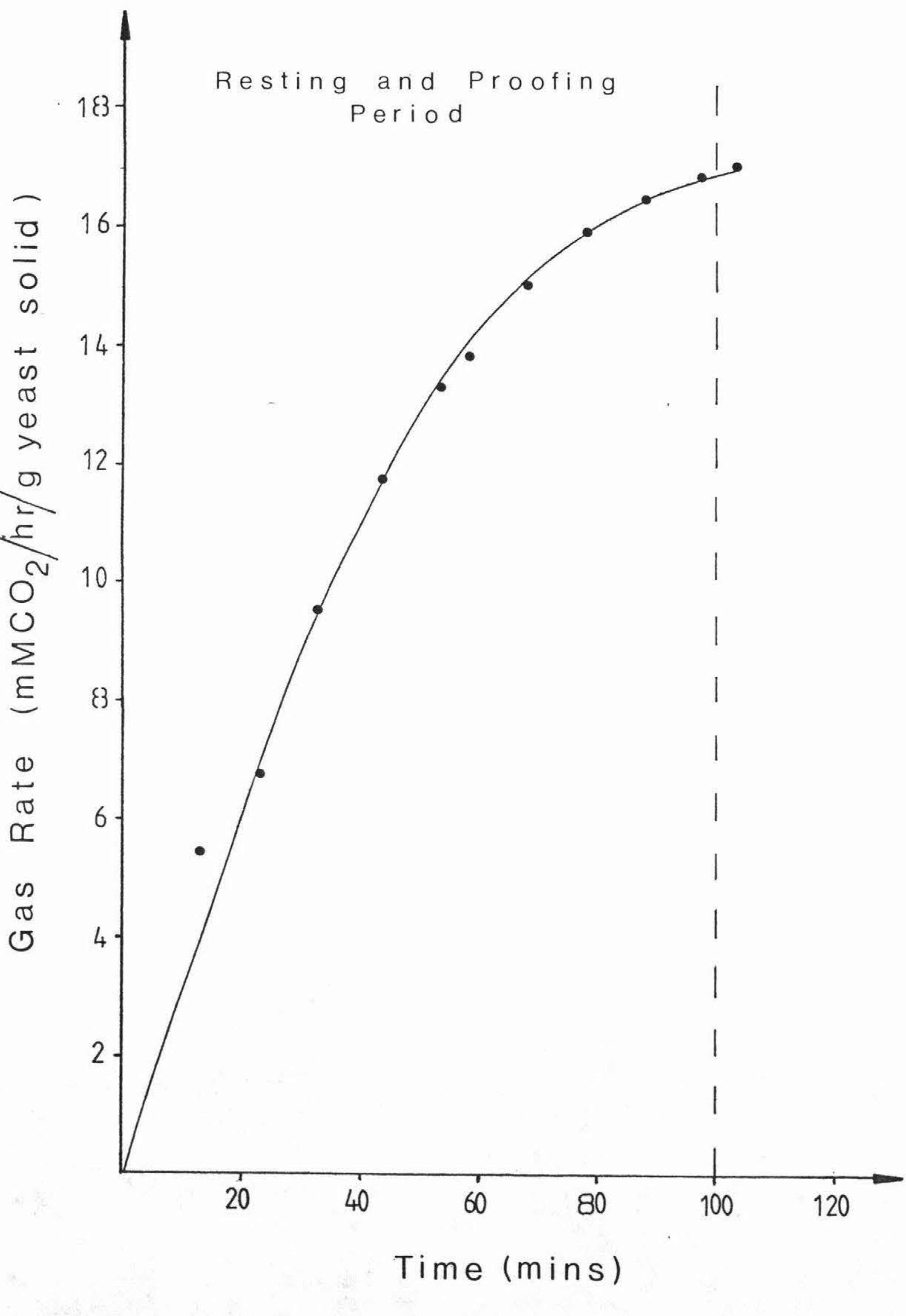
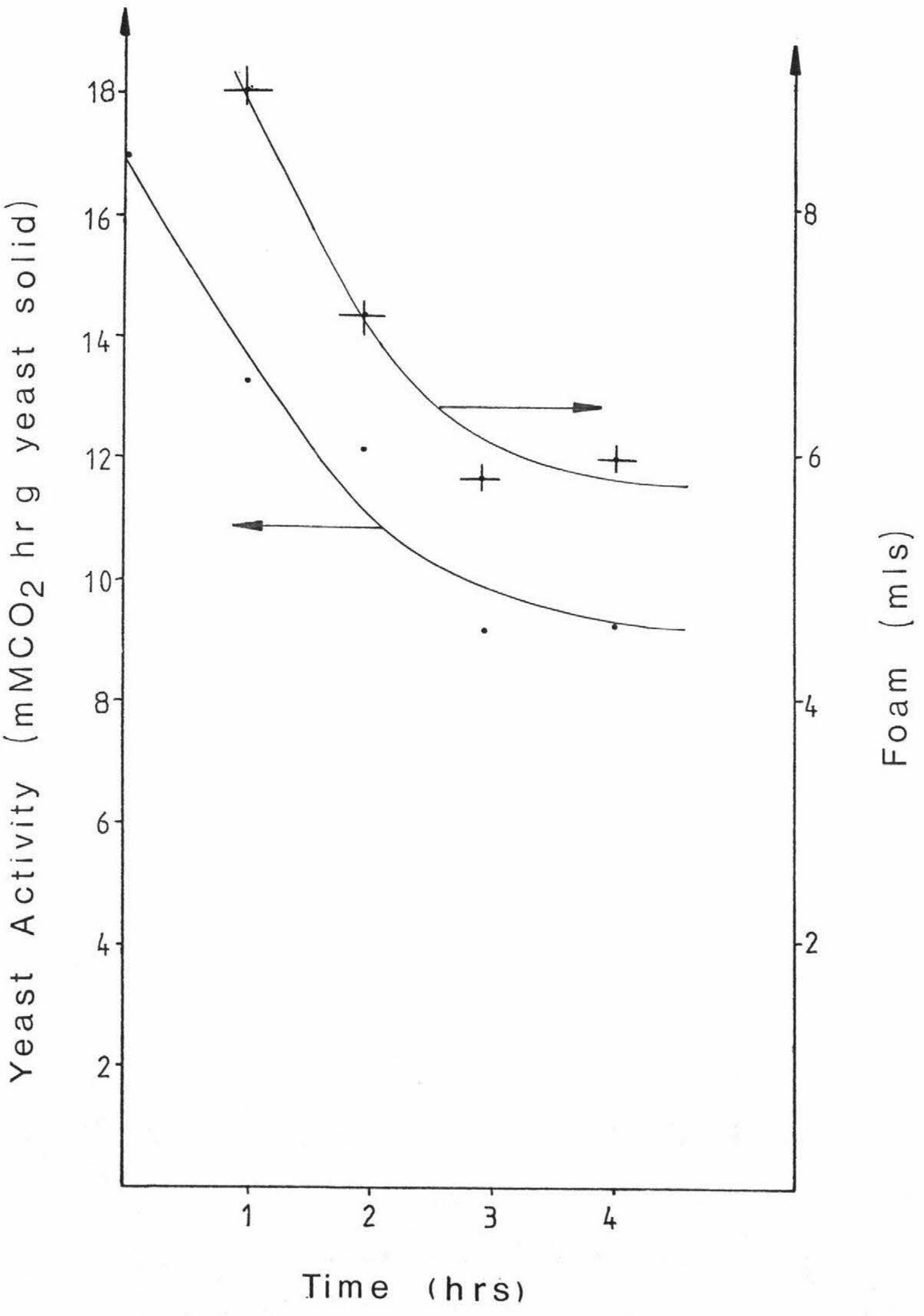


Fig.3.5 Gas Metabolic Activity and Foaming Activity During Cabinet Tray Drying at 40°C



the foam structure collapsed, resulting in an excessively low foam volume (Table 3.4). For this reason the foaming ability of Compressed Yeast samples taken at the start of the drying run were deleted. The differences in foaming ability between an active dry yeast and one with little activity is illustrated in Fig. 3.22.

Table 3.4 A Comparison of Yeast Activity and Foam Production During Rehydration to Determine the "Cut-off" Point for Low Activity Yeast.

Yeast Description	Activity (1)	Volume Foam Production (mls) 5	10 (min.)	15
ADY grown	8.7	5.9	6.6	7.2
and dried in				
the Laboratory	1.3	3.4	3.6	4.0
	1.2	3.0	3.6	3.6
Compressed Yeast	16.4	4.4	4.9	6.6
in a rehydration				
solution (2)	15.0	4.0	4.2	6.0

(1) $mMCO_2$/hr./g yeast solid

(2) Foaming was so vigorous that the foam structure collapsed.

3.1.6 Rehydration of Active Dry Yeast

The media in which ADY has been rehydrated can be classified into at least three categories (see section 1.1.5). These are salt based (2.5 - 10% $^w/v$), sugar based (1 - 2% $^w/v$), or distilled water. Employing the same batch of commercial ADY for these studies, rehydration was carried out at 40^{o}C. in 2.5 mls of test solution. The rehydrated yeast slurry was made up to volume using the standard suspending (salt) solution, and the activity tested as described in section 2.1.3. Table 3.5 lists the resulting activities.

Table 3.5 Effect of Rehydration Medium on Final Yeast Activity

Rehydration Medium (2.5 mls)	Activity (1)
salt solution (6.75% $^w/_v$)	8.4
distilled water	8.9
sucrose (2% $^w/_v$)	10.1

(1) mM CO_2/hr./g yeast solid

These results establish the importance of the nature of the rehydration reagent. It can be seen that the highest activity was achieved in 2% ($^w/_v$) sucrose and represents the degree of viability that can be obtained. The lower activities are still higher than endogenous respiration values. There may be no significant difference between observations for salt and distilled water suspensions of yeast cells.

3.2 Drying

3.2.1 Tunnel Tray Drying

The activity of Baker's Yeast, dried at differing relative humidities at 40°C., was determined. The influence of additives on drying parameters for yeast was also determined.

Drying curves of moisture content (dry weight basis) versus time were obtained and the resulting drying times presented in Table 3.6.

Table 3.6 Drying Times (hrs.) for Preparation of Active Dry Yeast at 40°C., at Indicated Relative Humidity (H_r) Values in the Presence (A+) and Absence (A-) of Additives (1).

H_r of tunnel air (2) @ 40°C.	Final Moisture Content of ADY (% dry weight)					
	9%		12%		14%	
	A+	A-	A+	A-	A+	A-
45%	24.5	48.0	10.0	16.0	7.2	11.0
31%	4.1	9.0	1.9	4.5	1.7	3.5
17%	3.6	7.0	1.9	4.0	1.7	3.0

(1) Additives, as described in section 2.2.1.

(2) Tunnel air speed 1.5 m/s.

The addition of an emulsifying agent and antioxidant (i.e. additives) halved the time to dry. Yeast dried with additives had an open crumbly structure while the absence of additives resulted in a hard brittle product. It was likely that this hardening of the yeast's surface was related to the absence of additives.

The relative drying time, θr defined as

$$\frac{\text{Time to dry with additives present}}{\text{Time to dry with additives absent}}$$

was found to be a function of humidity and the sample's final moisture content (Table 3.7).

Table 3.7 Relative Drying Times for Active Dry Yeast at 40°C., at Indicated Relative Humidity (H_r) Values.

H_r of tunnel air (2) @ 40°C.	Final Moisture Content of ADY (% dry weight)		
	9%	12%	14%
45%	0.51	0.62	0.65
31%	0.45	0.42	0.48
17%	0.51	0.47	0.56

(2) Tunnel air speed, 1.5 m/s.

A model was fitted using the MINITAB statistical computer programme.

$$\theta r = 0.452 + 0.103\ (H_r)^2 + 0.039\ (H_r) + 0.036\ (W_F)$$

where H_r = relative humidity at 40°C.

W_F = final moisture content.

The model's components are significant at ($p < 0.025$) for $(Hr)^2$ and ($p < 0.05$) for Hr.

As the activity of stored Compressed Yeast normally decreases with time the observed activity was adjusted to a uniform initial value for all experiments (see Appendix 2). The activity of the dried yeast was adjusted to set final moisture contents using standard curves (Fig. A 2.1). These corrected yeast activities are presented in Table 3.8.

The data of Table 3.8, which shows dried yeast activity at a range of final moisture contents and air humidities can be described by the model.

$$Ac = 11.53 - 2.233H_r + 1.87\,W_F - 2.867\,(H_r)^2$$

where Ac = dried yeast activity ($mMCO_2$/hr/g yeast solid)

H_r = relative humidity at 40^oC.

W_F = final moisture content.

All three terms are significant at ($p < 0.005$). A plot of activity versus relative humidity at 40^oC. (Fig. 3.6) shows an optimum humidity level, the curvature, becoming tighter as the moisture content decreases. Humidity control at 30% H_r would appear advantageous in the falling rate period of drying. The activity, is 14% higher at 31% H_r than at 17% H_r and 69% higher than at 45% H_r (from Table 3.8). The decline in activity, with time follows zero order reaction kinetics in the humidity range tested. This can be seen from Fig. 3.7 where the loss in yeast activity is expressed as a function of time and air humidity.

Table 3.8 Final Activity Values for Active Dry Yeast Preparations at 40^oC., at Indicated Relative Humidity Values and Final Moisture Content.

H_r of tunnel air @ 40^oC.	Final Moisture Content of ADY (% dry weight)		
	9%	12%	14%
45%	3.2	7.9	8.6
31%	10.4	12.1	12.5
17%	8.9	11.9	12.3

(1) Activity specified as mM CO_2/hr./g yeast solid.

From the drying curves of moisture content versus time further drying parameters can be determined. The resulting rate curves are presented in Figs. 3.8 - 3.10 and summarized in Table 3.9. A sample calculation is included for the 31% relative humidity run (Appendix 3).

In some situations the shape of the falling rate drying line can be related to the nature of the drying process (Perry, 1963). Using the procedures described (Perry, 1963), at 45% H_r the drying model is consistent with capillary action, and diffusion is required

Fig. 3.6 Dried Yeast Activity vs Drying Air Humidity at Indicated Final Moisture Contents

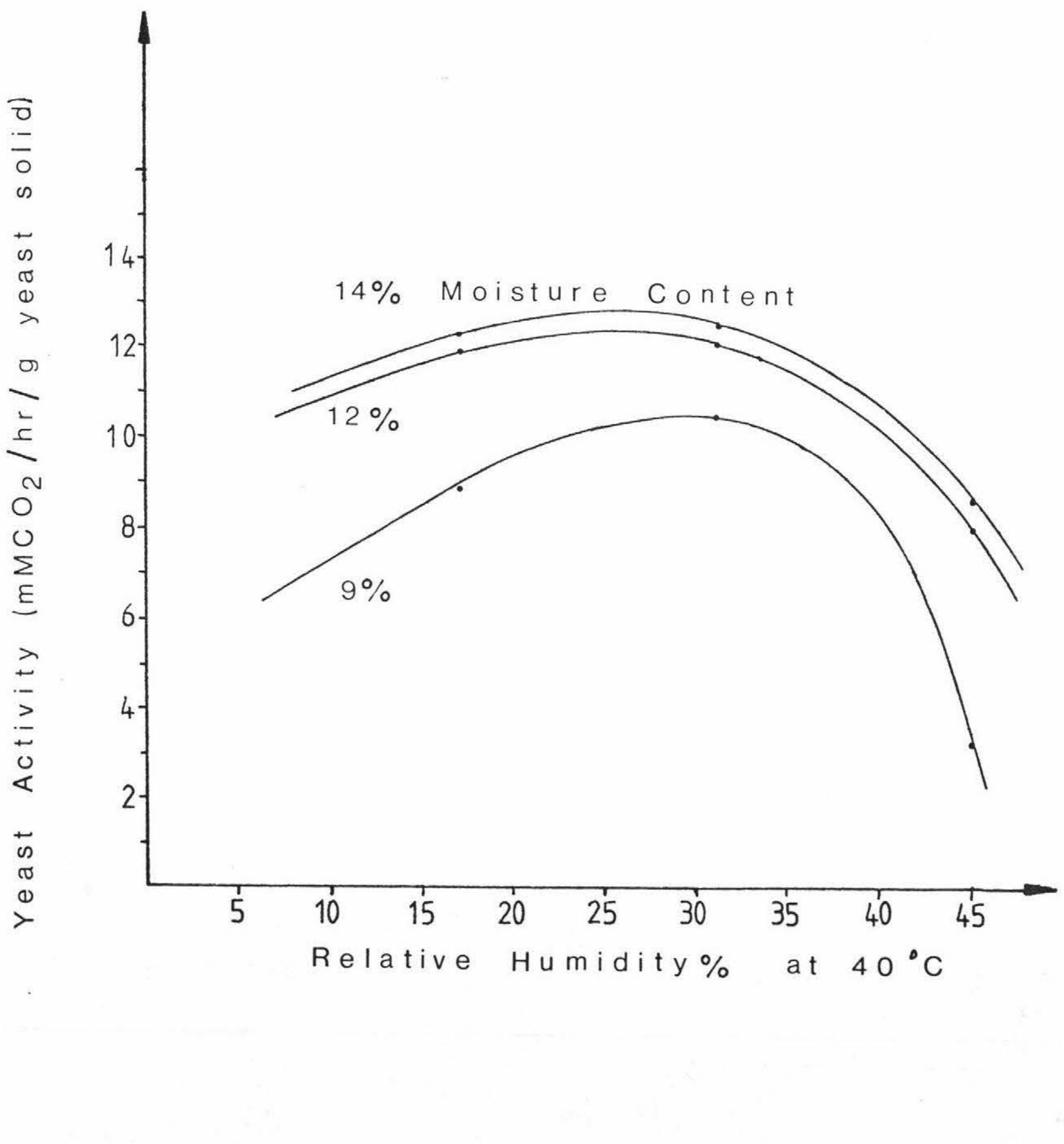
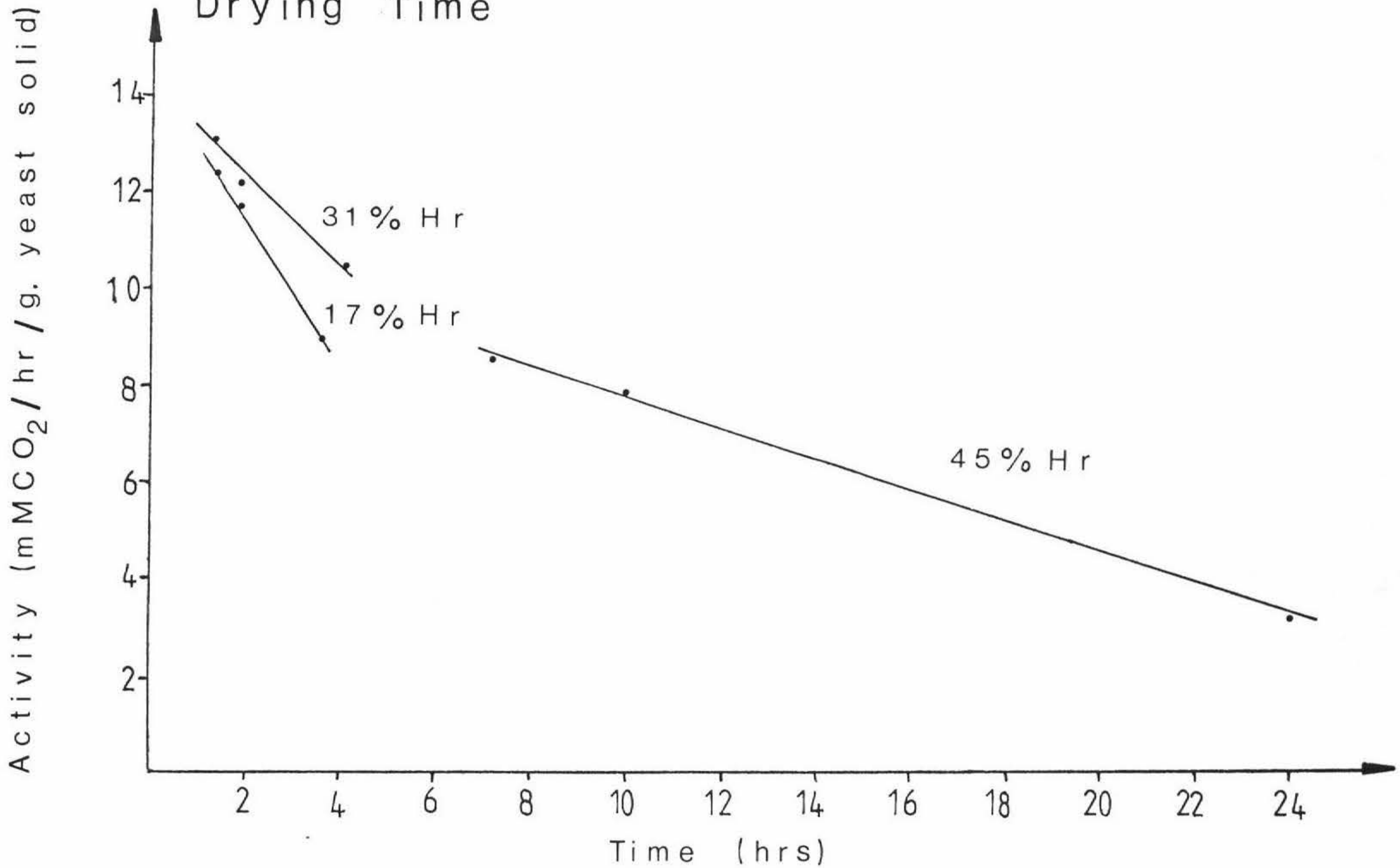


Fig. 3.7 Correlation between ADY Activity and Drying Time



to remove water in the pendular state. At 31% H_r the data best fitted the capillary model. At 17% H_r however, better fit was obtained using a two stage diffusion model. Using graphical differentiation techniques considerable error was involved. A greater collection of data would allow numerical differentiation and increased precision in determining the drying model. While a model relating dried yeast activity and drying time to drying conditions has been developed, the actual drying mechanism occurring can only be suggested. The drying parameters obtained are, however, a stimulus for further investigations in this area. For example, more data would need to be collected so that diffusion coefficients could be calculated.

Table 3.9 Summary of Drying Parameters for Drying Baker's Yeast at 40^{o}C.

Drying Parameter	Relative Humidity 17%	31%	45%
Constant Drying Rate x 10^{-3} (Kg/S/m^2 Kg dry product)	4.8	4.3	2.25
Falling Rate Period Characteristic			
a) 1st stage	diffusion only	capillary only	capillary
b) 2nd stage			diffusion
Critical Moisture Content (Kg H_2O / Kg dry Yeast)	1.56	1.12	1.36
Surface Heat Transfer Coefficient, hc (W/m^2 ^{o}C.)	26.1	23.3	12.1

3.2.2 Cabinet Tray Drying

Using the optimal humidity values determined in Section 3.2.1, i.e. 31% H_r @ 40^{o}C., a further trial was conducted to confirm that it was the emulsifying agent which changed the drying time of the yeast slurry. The interaction of drying temperature and emulsifier concentration, with drying time was determined at 31 - 32% relative humidity.

The concentration of antioxidant was maintained at the previously employed value of 0.13% (^{W}/W) and the emulsifier was present at 2% (^{W}/W), or absent. Compressed Yeast was dried to 9% moisture at

Fig. 3.8 & Fig. 3.9 Drying Rate of Compressed Yeast at 40 °C

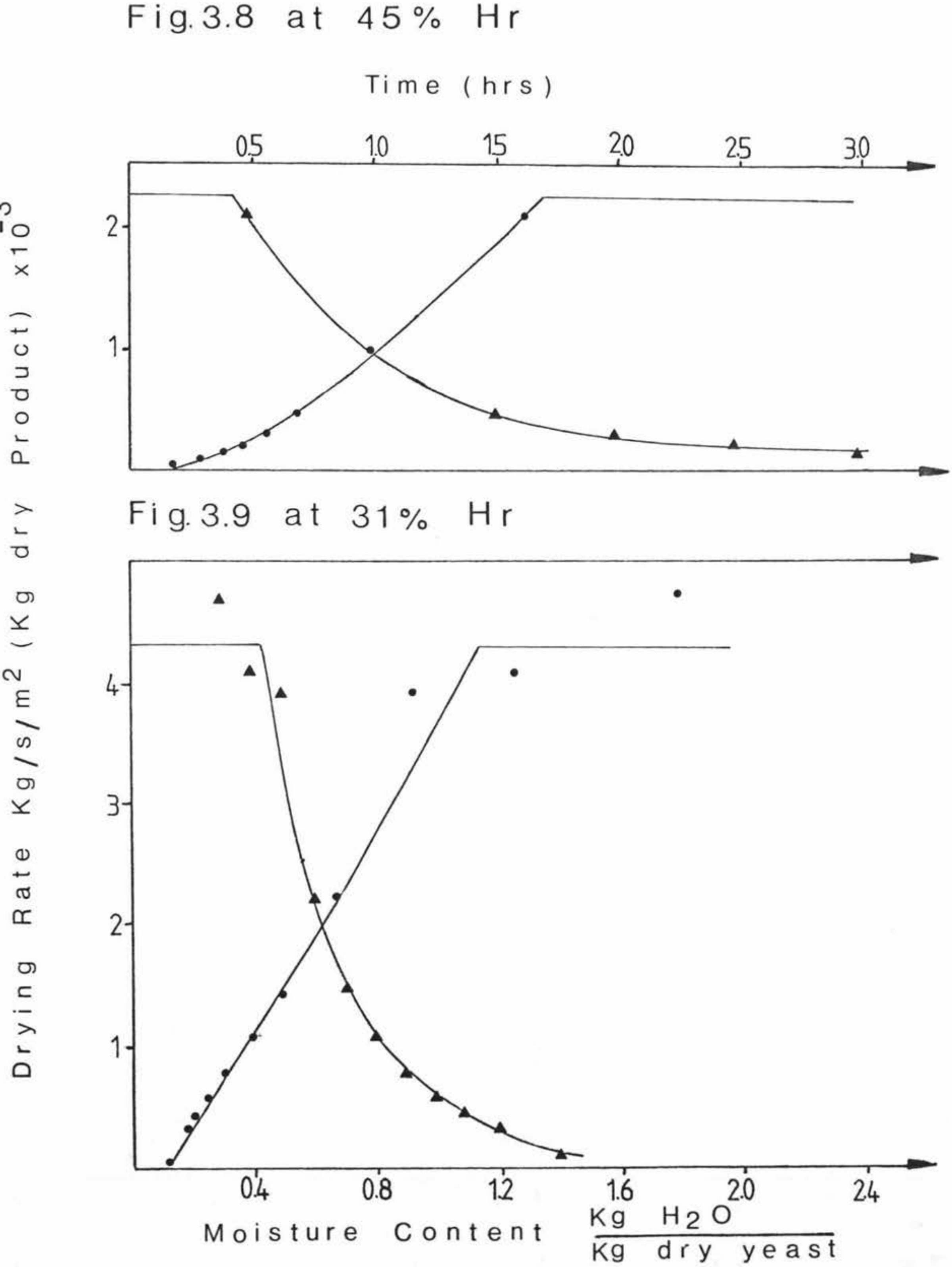
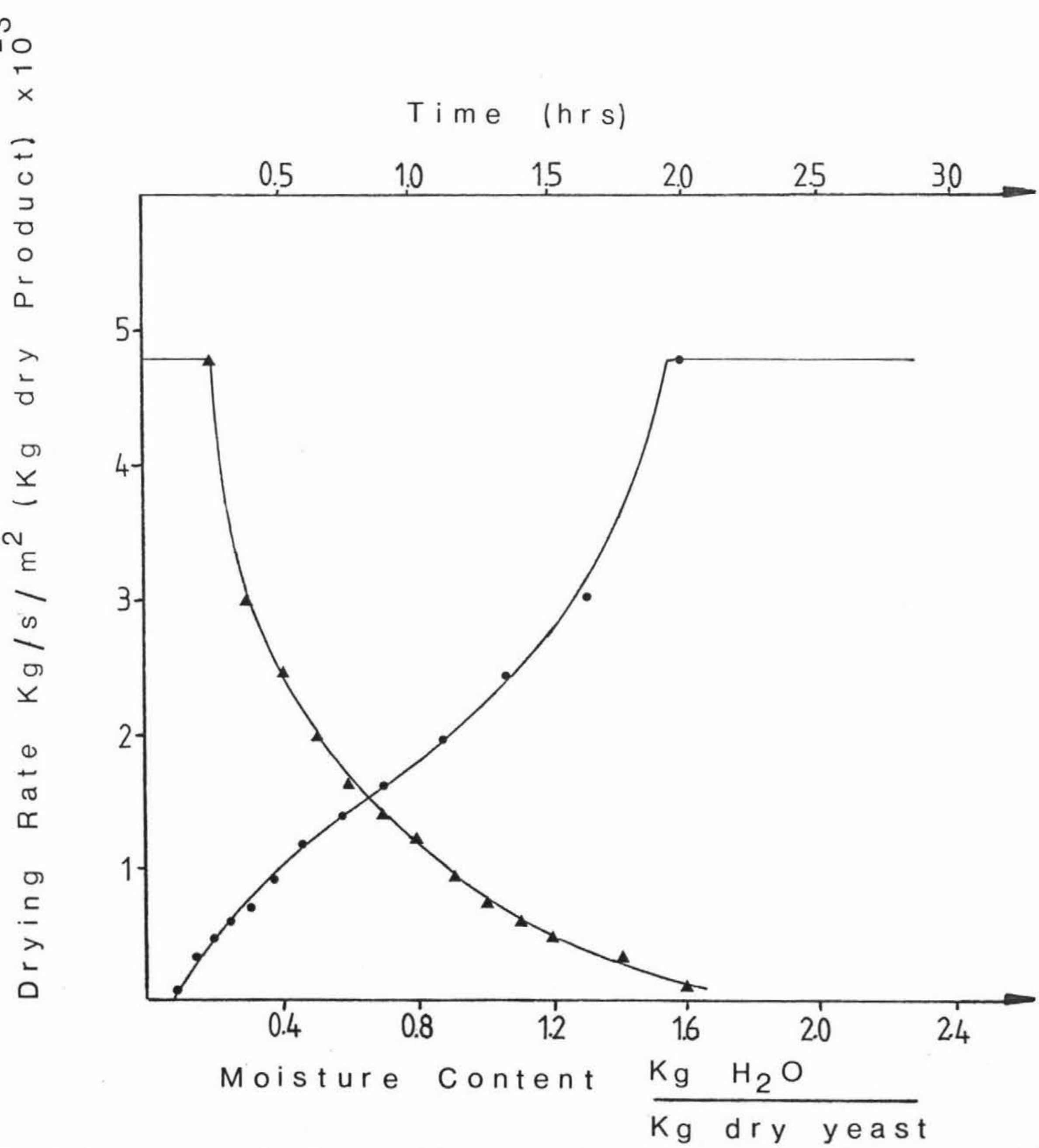


Fig. 3.10 Drying Rate of Compressed Yeast at 40 °C and 17% Hr



either $35^{o}C.$ @ 32% H_r or $40^{o}C.$ @ 31% H_r. A replicated 2^2 factorial design was used, which together with the dependent variable i.e. drying time is presented in Table 3.10. The data were analysed by multiple linear regression using the MINITAB statistical computer programme.

The generated model for drying time was

$$\theta = 6.753 - 0.73\,t - 1.622\,E\,M - 0.36\,E\,M \times t$$

where θ = drying time (hr)

t = temperature ^{o}C.

E M = emulsifier concentration

The drying time varied from 4 hours to 9 hours, however, the data variation in the trial without emulsifier at $40^{o}C$. is explained on the basis of an initially high moisture content.

The inclusion of an emulsifying agent was significant ($p<0.01$). The presence of an emulsifier in the yeast slurry appears to allow water removal to occur at a faster rate. This could be related to its detergent properties and a lowering of the surface tension. The open crumbly structure of dried yeast containing an emulsifier was characteristic.

The temperature change was significant ($p<0.1$) for a variation of $5^{o}C$. There was no interaction between the emulsifier concentration and the drying temperature. A plot of Residuals against the "Yield" showed that no data varied by more than 2 standard deviations from the fitted equation. The correlation coefficient, r^2 was 91%.

For the drier under consideration (hot air tray drying), the fitted equation allows the drying time to be determined for various temperature and emulsifier levels at a set humidity within the stated range of these variables.

Table 3.10 Effect of Temperature and Emulsifier on the Drying Time of Baker's Yeast

Temperature	Emulsifier	Interaction	Drying Time (hr)	
1	1	1	4.08	4.00
-1	1	-1	6.42	6.02
1	-1	-1	9.12	6.89
-1	-1	1	8.86	8.63

1 represents $40^{o}C$. @ 32% H_r and 2% ($^W/v$) emulsifier present.
-1 represents $35^{o}C$. @ 31% H_r and emulsifier absent.
Interaction is the product of Temperature and Emulsifier.

3.2.3 Other Drying Methods

In this section brief consideration is given to the possibility that other accepted methods for drying biological materials might be useful in the preparation of Active Dry Yeast. Acetone Extraction and Lyophilization were the methods considered.

Yeast activity and final moisture content of Active Dry Yeast produced from acetone extraction is presented in Table 3.11.

Table 3.11 Effect of Acetone Extraction on the Final Activity and Moisture Content of Baker's Yeast.

Yeast Temp. ^{o}C.	20	20	-20
Acetone Temp. ^{o}C.	20	-20	-20
Final Moisture Content	18.62	23.27	125.53
Yeast Activity (1)	1.1	0.8	3.3

(1) $mMCO_2$/hr./g yeast solid
Initial activity was 16.8 mM CO_2/hr/g yeast solids.
Initial moisture content was 265% (dry weight).

It is apparent that solvent extraction of Compressed Yeast was not successful. The lowest moisture content was obtained at room temperature. Freezing the yeast crumbs caused a reduction in the available surface area for solvent extraction and resulted in a raised moisture content. The highest activity obtained, a result of the high moisture content, was disappointing. A much higher activity was expected at the intermediate moisture level.

Freeze-drying would provide an alternative to high temperature drying. The decrease in moisture content and yeast activity with time were measured and plotted, see Fig. 3.11.

The time to reach 8% moisture was approximately 6 hours, a third longer than tray drying. Yeast activity decreased extremely rapidly between the 2nd and 4th hour while the moisture content was still at intermediate values (90 - 50%, dry weight basis). A scatter of measured values occurred towards the end of the drying period. This could be due to variations in moisture distribution throughout the crumbled yeast. If the Compressed Yeast were formed into a slurry and shell frozen on the flask's inner surface this problem would be reduced; additionally, various additives could now be included.

The initial activity of the yeast was taken to be that of Compressed Yeast frozen at the same time and under the same conditions as the material to be freeze-dried. The yeast was frozen at $0.6^{o}C./min.$ from $4^{o}C.$ to $-15^{o}C.$ and thawed at $0.2^{o}C./min.$ from $-15^{o}C.$ to $0^{o}C.$ Yeast frozen and then thawed at room temperature prior to activity measurements and under the above conditions lost little activity (15.8 mmoles CO_2/hr/g yeast solid) compared with fresh Compressed Yeast (17.0 units). This thawing rate would be different from the sublimation conditions for freeze-dried yeast and this could possibly be the basis of the high activity losses observed.

As these approaches to drying appeared to retain little of the initial yeast activity, no further work was performed on these two methods.

3.2.5 Isotherm Studies

Having determined that the relative humidity is an important drying parameter for maintaining ADY activity during production, the relative equilibrium humidity was determined. This would demonstrate the moisture equilibrium of dried yeast at $20^{o}C.$ Such information would be beneficial at the endpoint of drying and for ADY storage.

The moisture sorption isotherm for ADY is shown in Fig. 3.12. As there was no significant difference (t test, $p<0.05$) between ad-

Fig. 3.11(a) Activity Losses in Bakers Yeast During Lyophilization

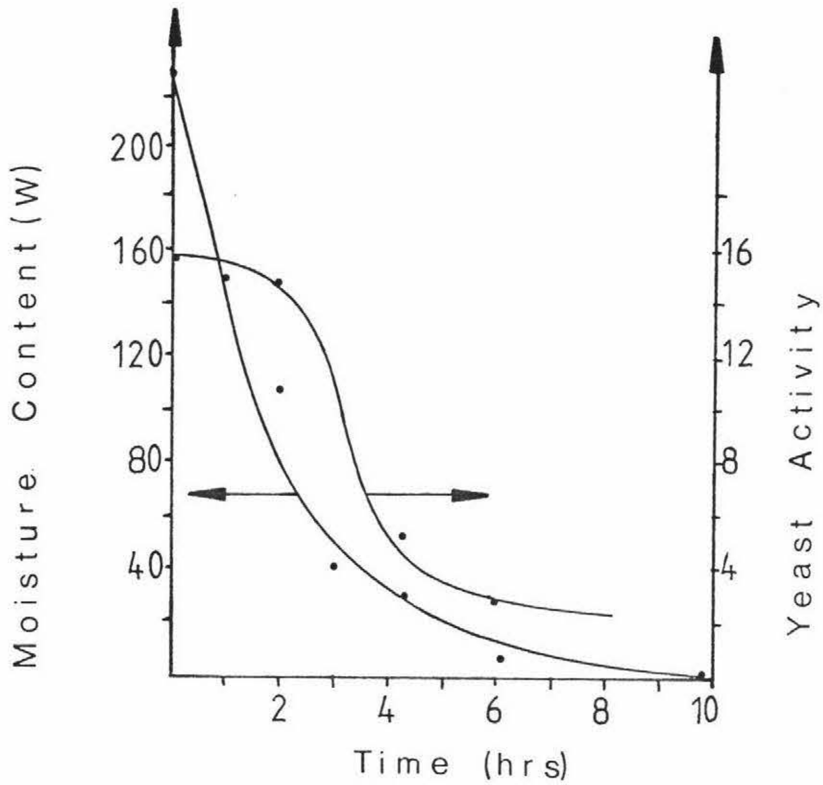


Fig. 3.11(b)

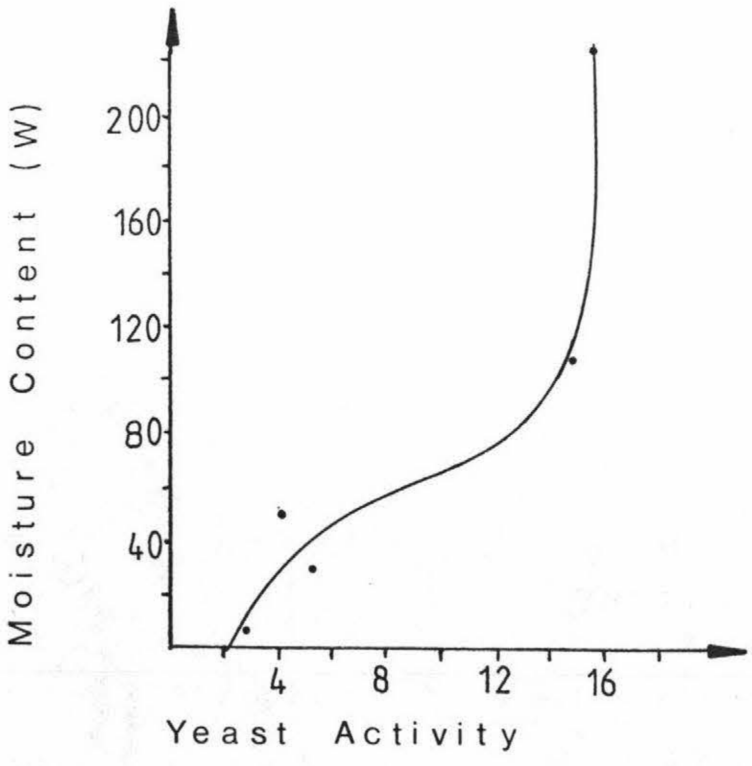


Fig. 3.12 Sorption Isotherm for A.D.Y.

at 20 °C

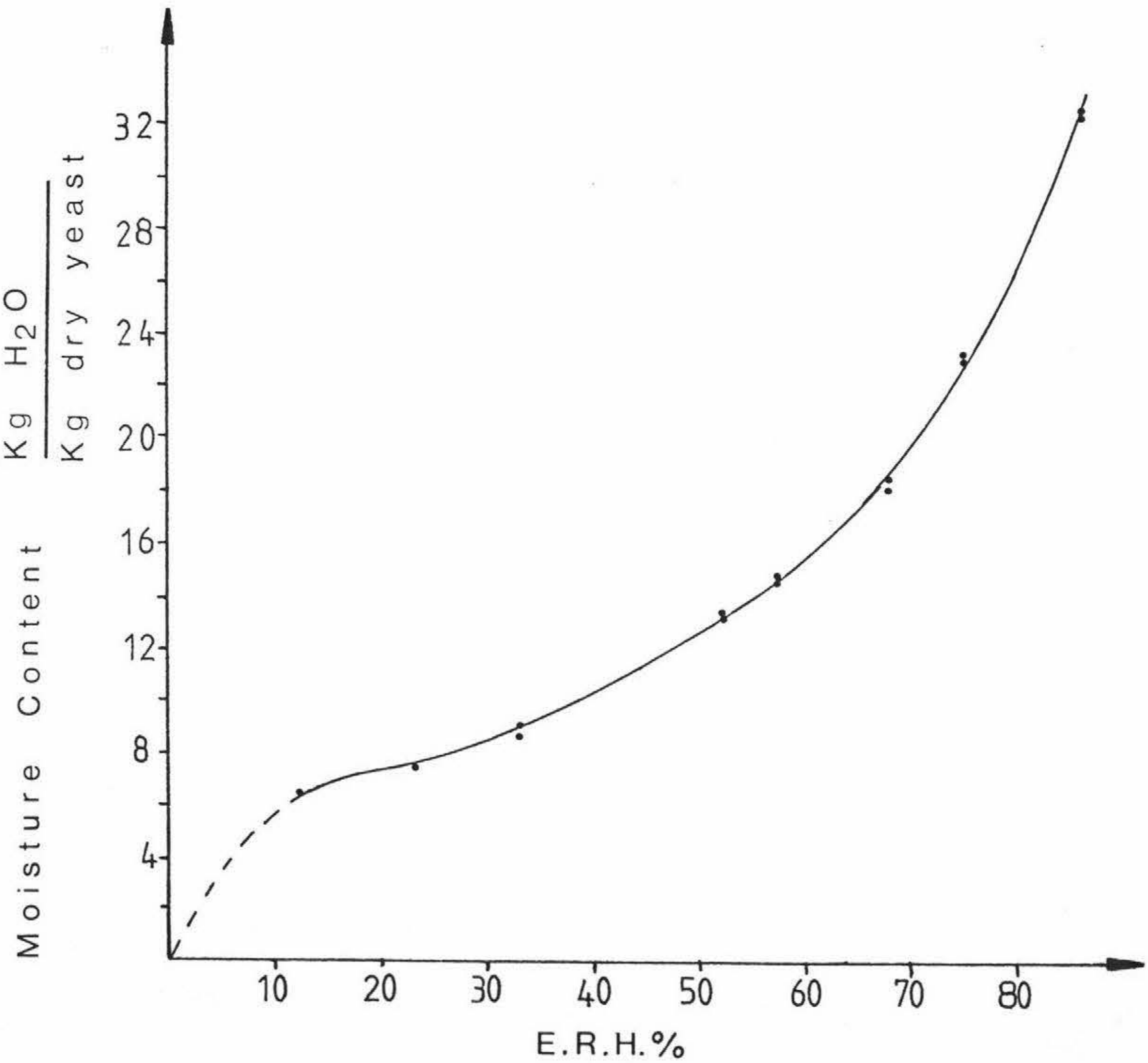


Fig.3.13 BET Monolayer Plot

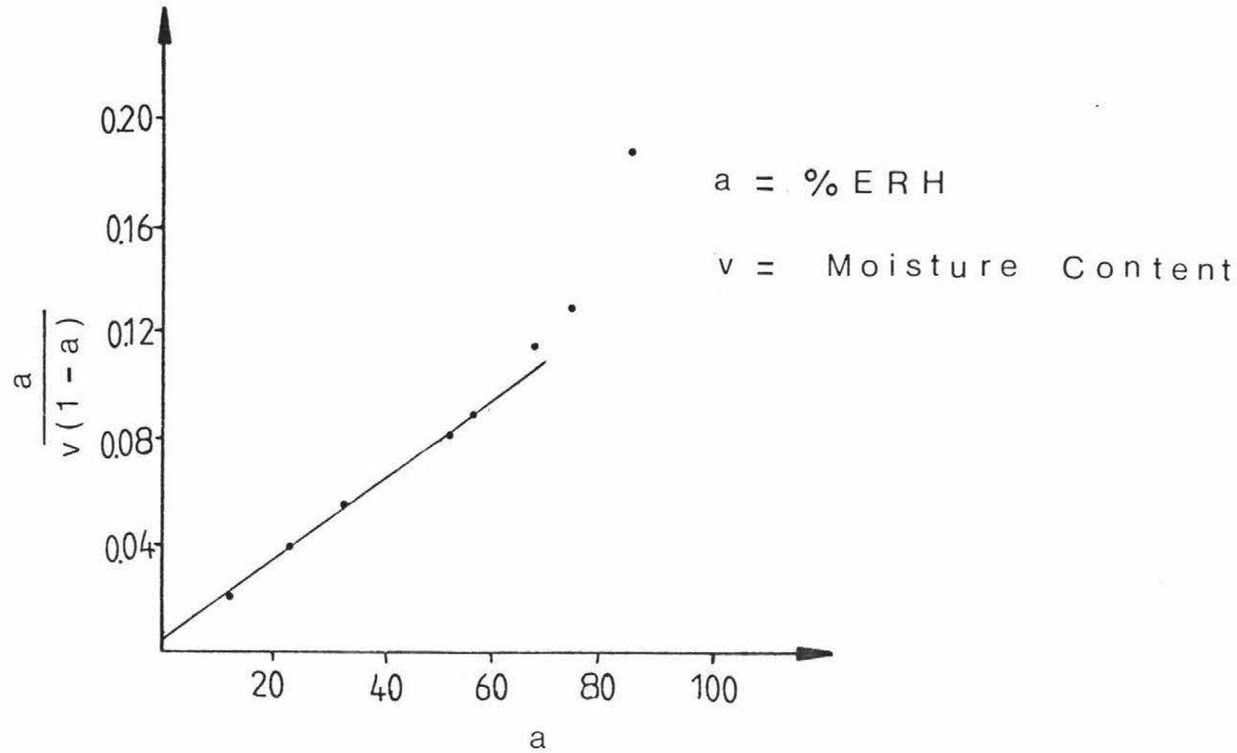
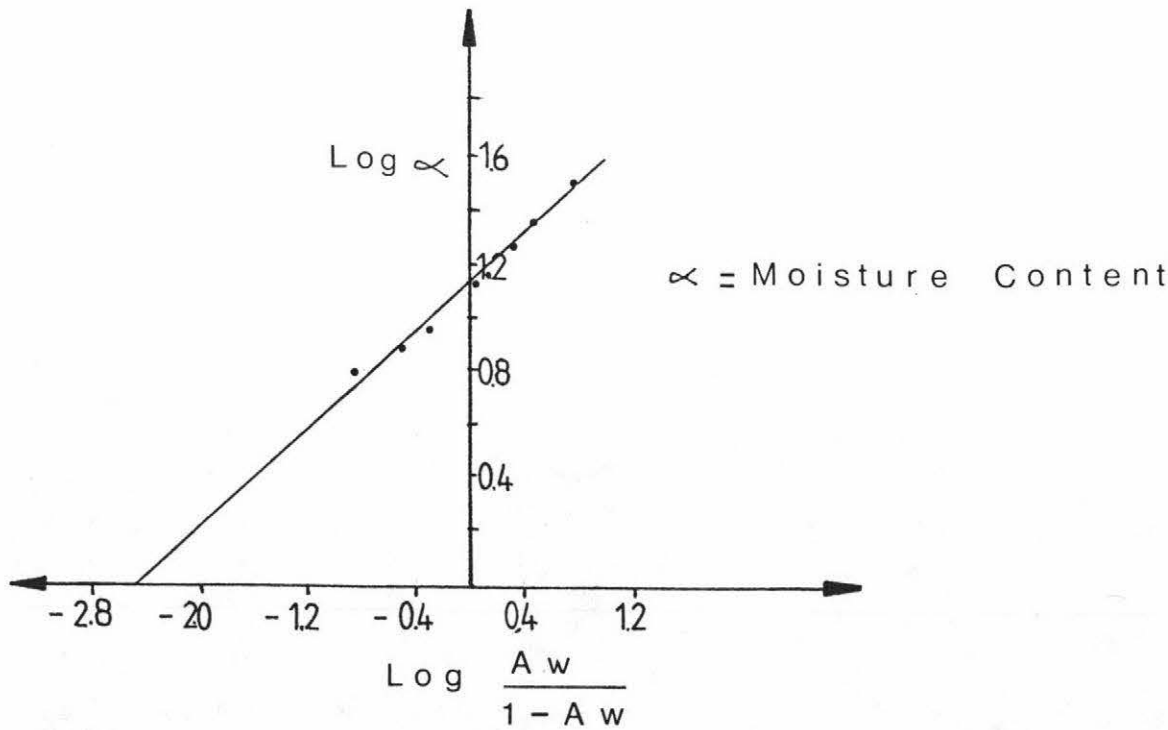


Fig. 3.14 Oswin (1946) Equation Plot



sorption and desorption values, the ADY tested did not exhibit hysteresis. Hysteresis is normally associated with the different energy requirements for filling and emptying capillary spaces during adsorption and desorption.

The data was fitted to the Brunauer _et al._ (1938), i.e. B E T equation and to the Oswin (1946) equation. The Oswin equation was found to fit many food isotherms, (Boquet _et al._, 1978) and the monolayer capacity (Vm) was determined as outlined by Labuza (1968). Fig. 3.13 shows a B E T monolayer plot.

The monolayer capacity was determined as 2.2% g H_2O/100 g dry substance (3% E R H). This was less than the inflection point (7% moisture and 15% E R H) normally regarded as the monolayer capacity (Labuza, 1975). A common drying endpoint, (8-9% moisture) corresponds to an E R H of 27-33%). Thus it appears possible to dry yeast to a much lower moisture level than is currently practised in factories. It was noted that the optimum drying conditions at 40^{o}C. were 30-32% H_r for yeast dried to 8-9% moisture. That is, the equilibrium humidity (or the humidity with which the samples' moisture content was in equilibrium) and the optimum drying air humidity were very similar.

The two parameter Oswin (1946) equation was fitted to the data. Fig. 3.14 is a plot to determine the constants in the equation. Using a χ^2 goodness of fit test ($\chi^2 = 0.49, \chi^2_{0.05,7} = 14.1$) there was no significant difference between the observed and predicted values. For dried yeast the moisture content of the package can now be calculated from

$$W = 13.49\left(\frac{Aw}{1-Aw}\right)^{0.454}$$

where Aw = Water activity, $\frac{ERH}{100}$.

3.3 Fermentation

3.3.1 Fermentation Conditions and Compressed Yeast Activity

The effects on yeast growth and metabolic activity of the environmental determinants viz. temperature, initial pH, dissolved oxygen, nitrogen and carbon concentrations of the medium were studied. The parameters, cell yield, Compressed Yeast activity, change in medium pH (Δ pH) and residual sugar were used to monitor the growth and metabolic activity.

Experimental Comment

A fractional fractorial design of resolution IV was chosen to screen the above variables. In a resolution IV design main effects are not confounded with each other, or with two factor interactions, though two factor interactions are confounded with each other. The design (2^{7-3}_{IV}) allows 7 factors to be estimated in 16 runs, as outlined in Appendix 4 and is termed a "fold over" design (Box and Hunter, 1961).

An empirical mathematical equation of the following general form can be fitted to the data.

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \dots \beta_i X_i + \varepsilon$$

where Y = Measured value of the response (dependent variable) e.g. cell yield, activity.

β_o = Constant

β_1 = Coefficient describing the linear response of the independent variable X.

ε = error.

Models were fitted to the fermentation parameters (cell yield, Compressed Yeast activity and residual sugar) using the MINITAB statistical computer programme. Initially a half normal plot, that is the magnitude of effect versus its half normal probability was used to gauge the significance of each term. Those terms departing from a straight line (or normal variance) were considered significant and further regression was performed. As the design is orthogonal, treatment coefficients did not change, but an increased residual allowed statistical tests to be conducted.

The Effect of Fermentation Conditions on Cell Yield

The cell yield, as seen in Table 3.12, varied during the 16 fermentation runs from 0.46 mg/ml to 4.6 mg/ml. From the half normal plot, Fig. 3,15 the carbon, dissolved oxygen, and the temperature values appeared to significantly affect the cell yield. The significance levels determined were carbon ($p<0.002$); dissolved oxygen ($p<0.002$); and temperature ($p<0.01$). The model best fitted to the data was:

$$Y = 2.038 - 0.356X_1 - 0.873X_2 + 0.779X_3$$

where the coded variables (see Table 2.9) are

X_1 = Temperature

X_2 = Carbon concentration

X_3 = Dissolved oxygen

Data transformations had little effect on the fitted model e.g. $\sqrt{x}$ raised r^2 (the correlation coefficient) from 90.5% to 93.0% and $\ln x$, $\log x$ to 92.0%, though x^2 and $\frac{1}{x}$ lead to a decrease. A residual plot (the difference between the observed data and the values predicted by the equation) were randomly distributed between values of -2 to 2 when plotted against cell yield, Fig. 3.16. A residual plot is used to identify observations that are remote from the majority of the data (2 standard deviations) or far from the fitted equation.

While it is recognized from literature that growth yields of micro-organisms will be affected by temperature, dissolved oxygen and carbon substrate concentration, the above confirmation of accepted microbial behaviour can be contrasted with aspects of the next section on yeast activity where it is shown that this kind of classical response is not paralleled.

Table 3.12 Fermentation Response Data for Strain F.I.1, Grown Under Various Conditions in the Laboratory and Assayed as Compressed Yeast at Cell Harvesting.

Run No.	cell yield mg/ml	activity (1)	final pH	ΔpH	Residual Sugar (2)	Carbon/ Nitrogen ratio
1	2.430	17.6	3.48	0.52	0.9	6.38
2	1.360	10.9	3.65	0.35	0.4	1.78
3	0.565	19.2	5.60	0.40	0.5	1.78
4	3.520	3.3	4.45	1.55	0.2	6.38
5	4.125	14.9	2.86	1.14	0.5	14.46
6	0.460	15.4	3.55	0.45	0.5	4.04
7	1.910	16.2	5.85	0.15	0.8	4.04
8	0.985	3.7	4.70	1.30	44.5	14.46
9	1.905	17.1	3.80	0.20	0.5	1.78
10	1.460	9.2	3.50	0.50	6.0	6.38
11	4.670	13.5	4.80	1.20	0.6	6.38
12	0.625	16.8	5.48	0.52	0.6	1.78
13	0.575	19.2	3.65	0.35	0.2	4.04
14	3.125	6.5	2.98	1.02	0.4	14.46
15	2.970	16.1	4.20	1.80	0.6	14.46
16	1.915	14.4	5.40	0.60	0.6	4.04

(1) $mMCO_2$/hr./g yeast solid

(2) mg/ℓ. As the sugar analysis is $\pm$ 4 mg/ml, only runs 8 and 10 can be regarded as having residual sugar.

Table 3.12 continued, Fermentation Response Data for Strain F.I.1

Run no.	Ys (1)	Colour (Munsell Notation) hue %	value	chroma	ADY Foaming Test (4)
1	14.6 An (2)	17.5	5.0	6	4.5
2	49.1	20.0	5.5	6	3.1
3	20.4 An	22.5	9.0	6	3.0
4	21.1	20.0	7.0	9	3.0
5	24.8	30.0	6.0	6	3.1
6	16.6 An	15.0	3.0	4	3.3
7	68.9	20.0	7.0	6	4.0
8	5.9 An (3)	15.0	3.5	4	3.1
9	68.8	20.0	5.0	4	3.3
10	8.8 An (3)	15.0	4.0	4.5	3.0
11	28.1	20.0	7.5	6	3.2
12	22.6 An	22.0	6.0	4	3.3
13	20.7 An	20.0	6.5	6	3.2
14	18.8	17.5	4.5	6	3.0
15	17.8 An	21.0	7.0	4	4.5
16	69.1	21.0	7.0	5	3.3

(1) Yield Coefficient, Ys, expressed as g dry weight of cells/mole of energy substrate used.

(2) Fermentations not agitated and regarded as anaerobic (An).

(3) Residual sugar present.

(4) ADY Foam test after 15 min., reject value set at 5 mls and below (see Section 3.1.5.)

Fig. 3.15 Half Normal Plot of Cell Yield

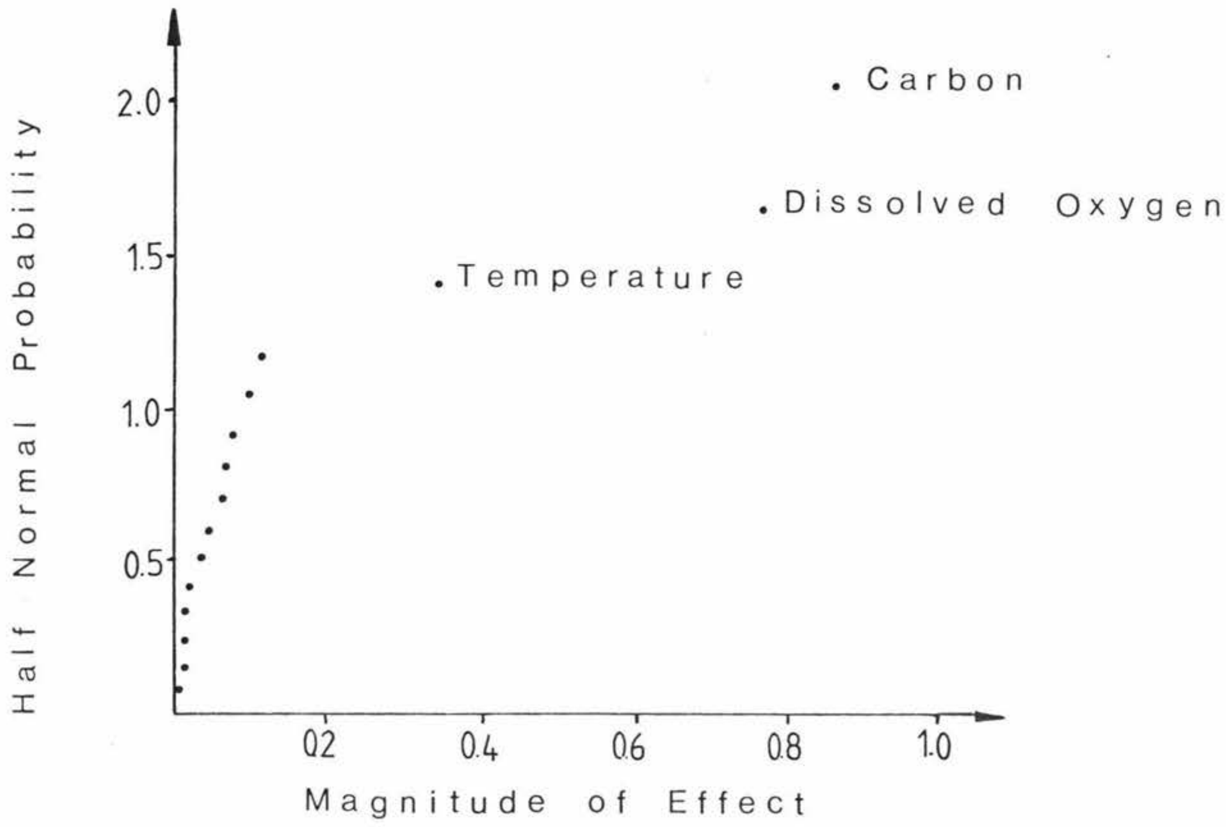
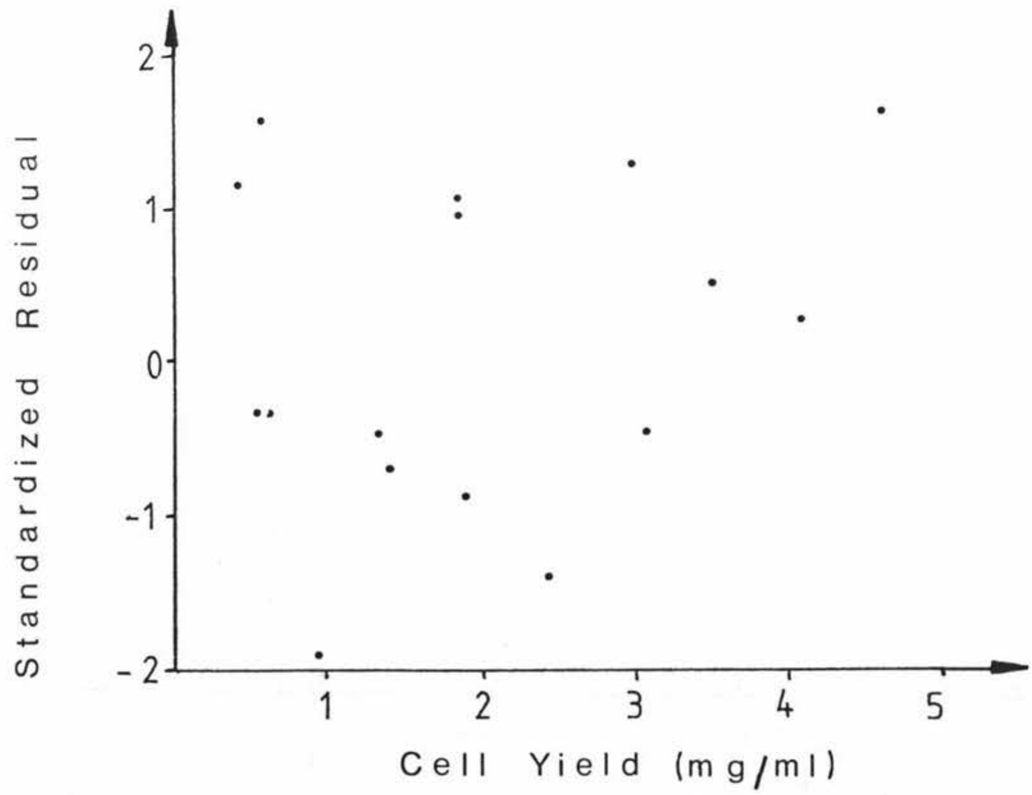


Fig.3.16 Plot of Residual verus Cell Yield



Response of Yeast Activity to Tested Environmental Conditions

Compressed Yeast activity ranged from 3.3 to 19.2 $mMCO_2$/hr./g yeast solid over the range of fermentation conditions examined, Table 3.12. The temperature, carbon concentration and dissolved oxygen appear as factors significantly affecting the final activity of the harvested yeast, Fig.3.17. The significance levels were, for temperature ($p < 0.002$); for carbon ($p < 0.002$); and for dissolved oxygen ($p < 0.1$). A model was fitted to the data of the form:

$$Y = 41.54 - 0.746X_1 - 2.224X_2 - 1.265X_3$$

where the decoded variables were

X_1 = Temperature ^{o}C.
X_2 = Carbon level (% (^{W}/v) glucose)
X_3 = Dissolved oxygen agitated (1) or standing (-1)

The model developed explains 82% of the observed variation in the data , 89% using an χ^2 transformation. This suggests that other factors, not included in this study also contribute to activity. However these factors and their significance fell outside the present study. A residual plot, Fig. 3.18 shows one spurious datum point.

While the three factors (X_1, X_2, X_3 above) were important to the observed values of both cell yield and yeast activity, their influence varied according to the design level to which they were assigned e.g. high or low. Best cell yields and activity occurred at the lower temperature level chosen, viz. 28^{o}C. However, yeast activity was highest at low concentrations of carbon and dissolved oxygen while cell yield was greatest at high concentrations of carbon and dissolved oxygen.

The investigation has shown that the optimum conditions for achieving cell yield (a commercially important consideration) were not the optimum conditions for developing a yeast with the greatest fermentative capacity. While there was a single optimum temperature for both maximum cell production and activity, the optima for substrate and dissolved oxygen differed significantly.

pH of the Fermentation Medium

The pH is a common parameter in fermentation studies, however it

Fig. 3.17 Half Normal Plot of Activity

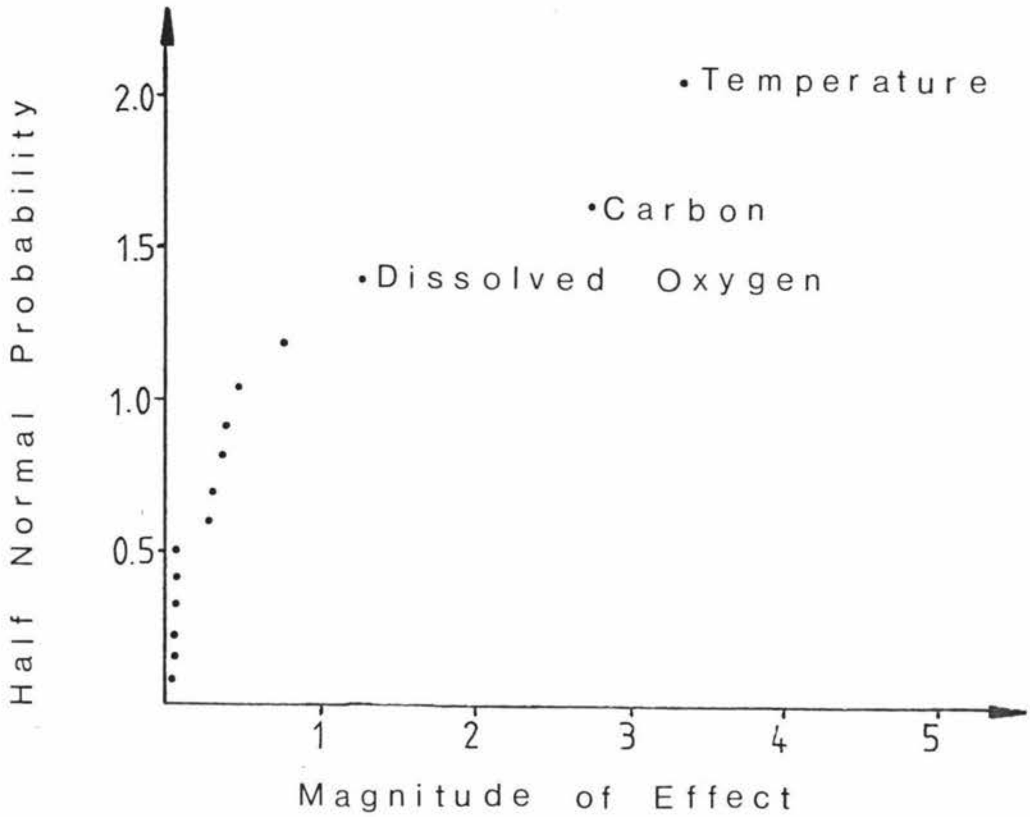
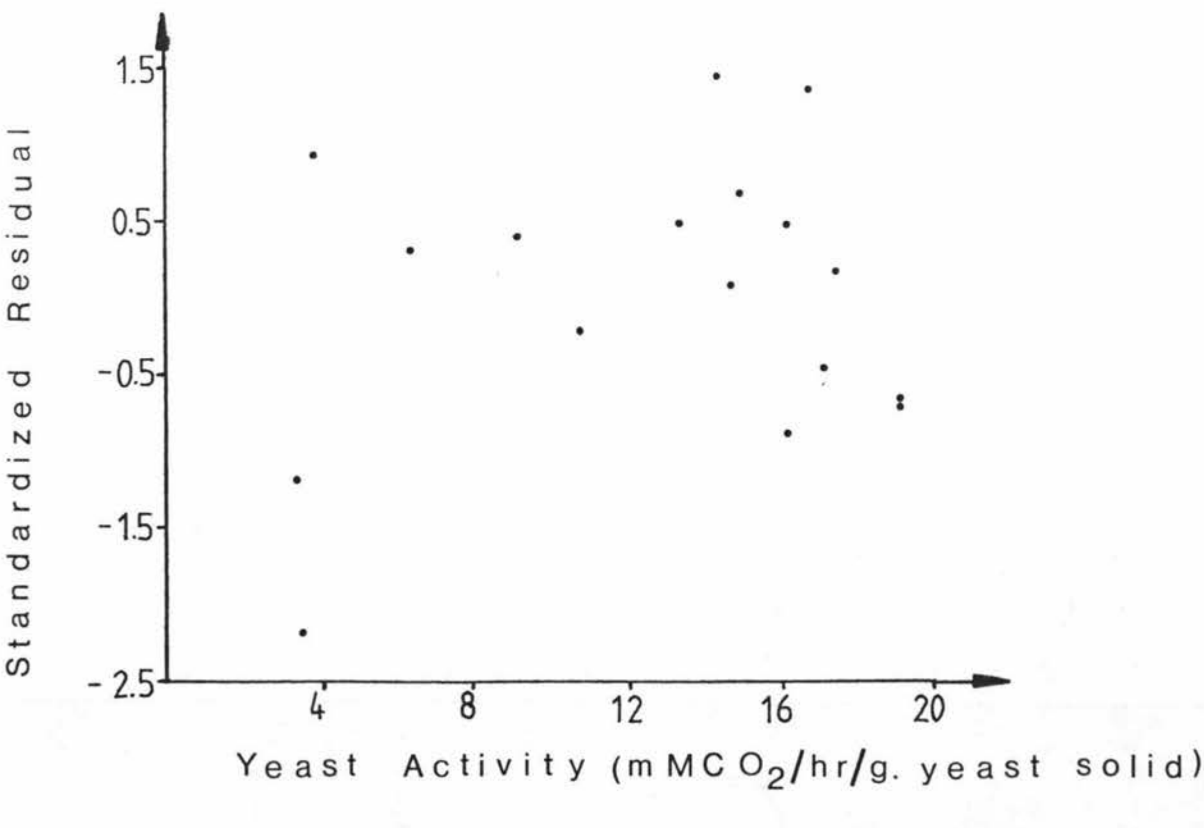


Fig. 3.18 Plot of Residual vs Yeast Activity



has been shown above that the initial pH of the medium does not significantly affect the yeast's activity. Neither is there any correlation between the changing pH (ΔpH) of the medium, resulting from culture growth, and yeast activity. Fermentation parameters which significantly contributed towards the magnitude of Δ pH were found to be the carbon and nitrogen concentrations and the initial pH value. As pH did not appear to influence Compressed Yeast activity, mathematical models were not developed.

Residual Sugar in the Spent Fermentation Broth

It can be seen from Table 3.12 that except for two fermentation runs (numbers 8 & 10) all glucose was utilized during yeast growth. The media would have become substrate limiting and the Yield Coefficient, Ys, can be expressed as g dry weight of cells/mole of energy substrate used.

For the standing (anaerobic) fermentation the average yield coefficient approached 19 (deleting the two runs with residual sugar). The Ys values, determined for the agitated experimental runs, appeared to vary considerably.

Dried Yeast Colour

The growth medium was shown to affect the colour of the dried yeast. The dried yeast was compared with standards which represent equally spaced divisions of the three attributes of colour known in the Munsell System of Colour Notation as hue, value and chroma (Munsell, 1960). The hue (its relation to red, yellow, green, blue and purple) the value (its lightness) and the chroma (its strength) were determined and are presented in Table 3.12.

It can be seen that the growth temperature affected the hue and chroma components of the dried yeast colour (half normal plots, Figs. 3.19, 3.20). Dissolved oxygen also significantly affected the hue and likewise the initial pH significantly affected the chroma. The value component appeared unaffected by fermentation conditions. Colour is a parameter considered by the yeast manufacturer and it was shown here that the final colour was a function of temperature, dissolved oxygen and initial pH. Substrate concentration, viz. carbon and nitrogen, did not affect the final yeast colour.

Fig. 3.19 Half Normal Plot of Chroma Component of ADY Colour

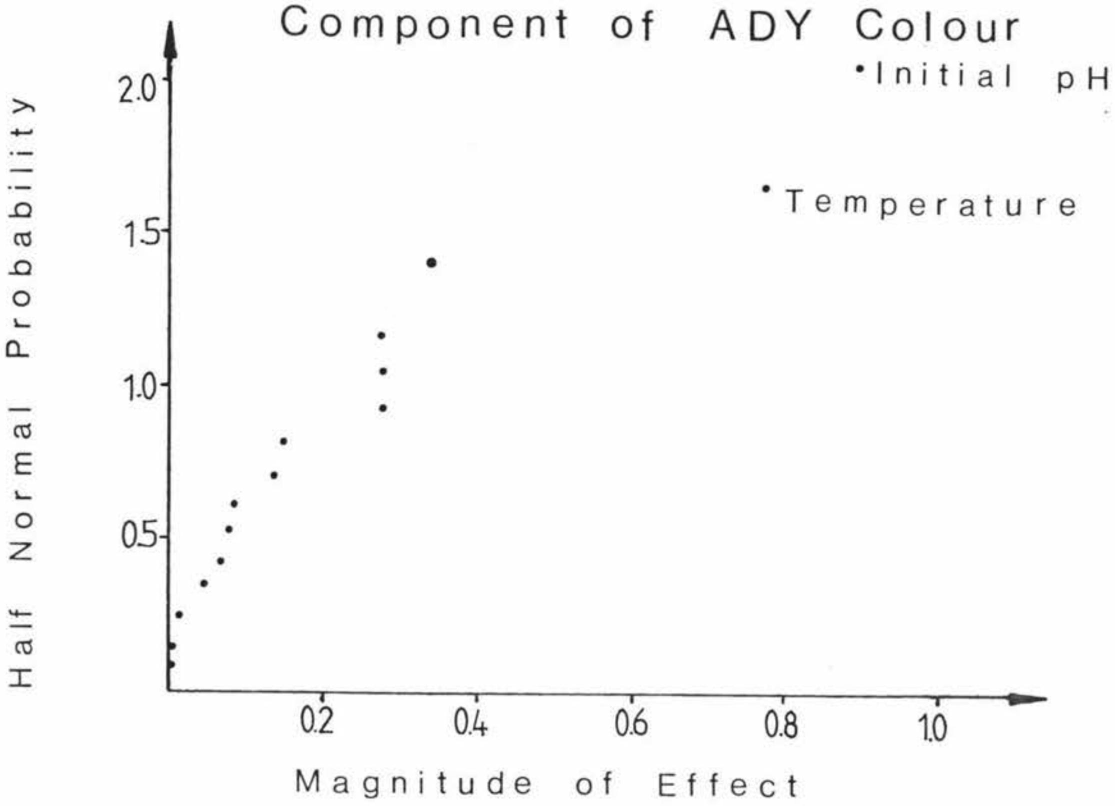
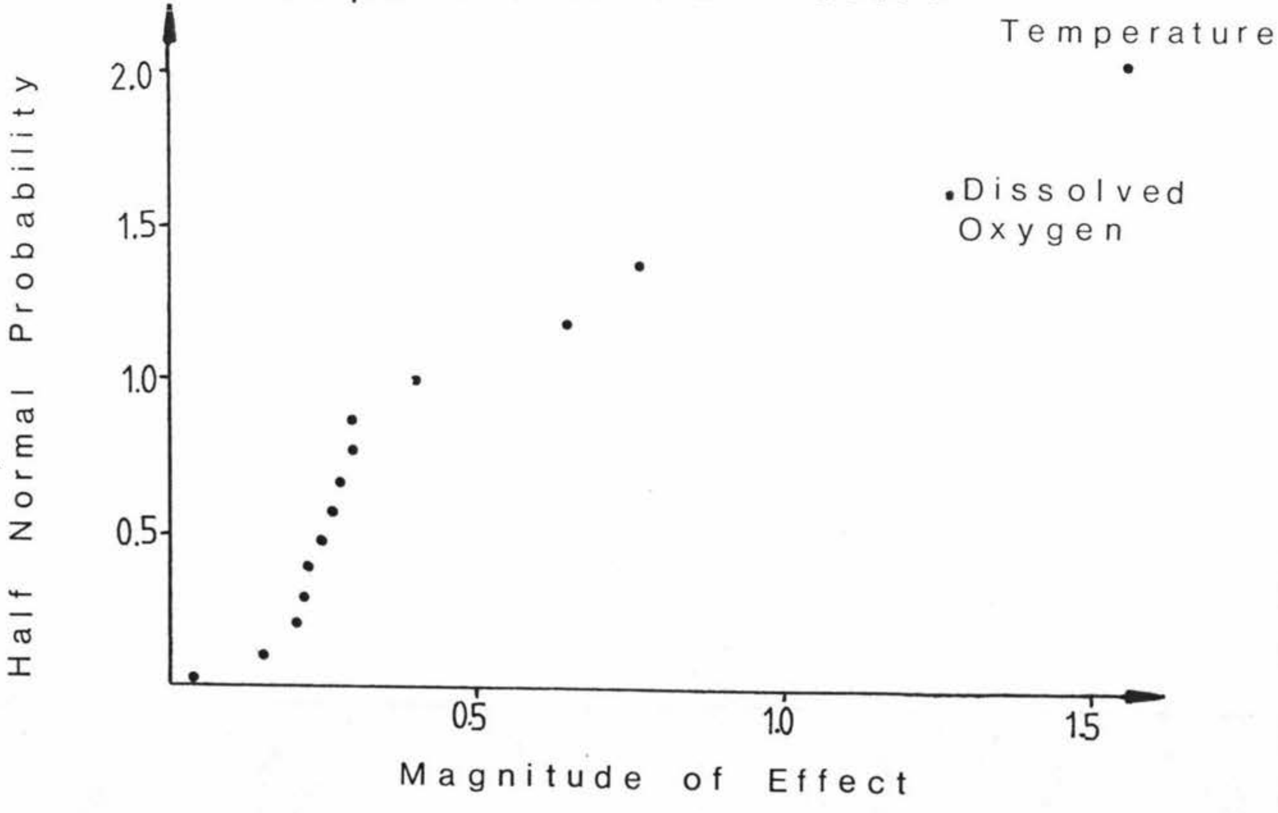


Fig. 3.20 Half Normal Plot of Hue Component of ADY Colour



Carbon and Nitrogen Ratio of the Media

The variations in glucose and nitrogen concentrations in a given medium can be expressed as a carbon to nitrogen ratio. As shown in Table 3.12 there was no correlation existing between the four C/N ratios examined and the observed activities of the Compressed Yeast.

3.3.2 Growth Media and Dried Yeast Activity

The Compressed Yeast samples obtained from the 16 fermentations of section 3.3.1 were dried using the cabinet tray drier at standard conditions, as outlined in section 2.2.2. The 16 ADY samples showed negligible foaming ability when rehydrated. Foam volume in each case was below the arbitrarily chosen reject value, Table 3.12. This dramatic loss in activity could now be related to either cultural or environmental factors as outlined below.

Cultural Factors

By chance, a heat sensitive yeast may have been selected during the original isolation programme of F.I.1. For this reason fresh commercial Compressed Yeast was used as an inoculum. The fermentation conditions of trials 5 & 7, from the 16 examined previously, were selected on the basis of their yeast activity and cell yield to be repeated with a Compressed Yeast inoculum.

From Table 3.13 it can be seen that there was a decrease in activity and cell yield when using the commercial culture. This may have been related to the change of medium from molasses to a synthetic one. The activity of ADY however was still negligible.

Environmental factors contributing towards this phenomenon were sought.

Environmental Factors

The composition of the yeast growth medium was now examined, in order to relate it to yeast activity at both the compressed and dried stages. Firstly, Compressed Yeast and ADY were produced from molasses, the commercially used medium and secondly a sequential medium was developed as described in Section 2.3.1.

Table 3.13 Effect of Inoculum Type and Growth Medium on Yeast Activity

	Cell Yield (1)	Yeast Activity (2)		
		inoculum	Compressed	ADY
Cultural Type.				
1. Basal, F.I. inoculum				
Run 5	4.125	-	16.2	Fail (4)
Run 7	1.910	-	14.9	Fail
2. Basal, Compressed Yeast inoculum				
Run 5	3.830	15.8	14.7	Fail
Run 7	1.580	15.8	10.9	Fail
Environmental				
3. Molasses	6.260	16.4	8.8	1.1
Molasses + Basal (3)	6.410	16.8	15.8	1.3
4. Peptone (5)		16.8	14.5	11.3
Peptone + Basal		16.4	14.6	8.7
Peptone + Basal + 3% glucose		16.4	11.5	1.2
Peptone + Basal + 0.5% glucose		16.4	14.8	1.3

(1) cell yield mg/ml

(2) Yeast activity $mMCO_2$/hr./g yeast solid.

(3) Basal medium minus glucose.

(4) Dried yeast sample failed foaming test.

(5) 0.5% Peptone Water (Oxoid, C M 9).

Molasses used alone as a medium produced a high cell yield. However the yeast activity was increased considerably (8.8 mmoles CO_2/hr./g yeast solids to 15.8) by the addition of vitamins and minerals contained in the basal medium, excluding glucose. As shown by Table 3.13, the ADY produced from these batches of cells again had negligible activity. Thus it was demonstrated that yeast grown in the laboratory on a commercial molasses medium, although possessing high activity after compressing, lost virtually all of this upon drying.

When using the sequential medium, the inoculum for each run was commercial Compressed Yeast and as the quantity of inoculum varied slightly, the growth was monitored by the presence of budding cells and change in culture pH. With a peptone base medium there was a small loss in activity during each processing stage, Table 3.13. It was unlikely that any yeast growth had occurred. The absence of budding cells, the presence of gram -ve and gram +ve cocci from the Compressed Yeast inoculum (indicating no growth competition from yeast) and virtually no decrease in medium pH (7.0 to 6.8) attested to the failure of the yeast to develop. Adding the basal medium (without glucose) to the peptone stimulated growth. Approximately half the cells were budding after 24 hrs. fermentation and the pH had decreased to 5.7. The effect of these supplements on the activity of Compressed Yeast was slight (14.6 $mMCO_2$/hr./g yeast solid) but reduced the ADY's activity to 8.7 units. Finally, adding glucose to the medium eliminated the gas producing ability of dried yeast. Growth had been stimulated further, this being evident by the high proportion of budding cells and the decreasing pH (to 5.5 for 0.5% glucose and 3.55 for 3% glucose). In Fig. 3.21 three doughs are shown. First, dough leavened by a poor dried yeast, second by a yeast with moderate activity after drying and finally by a dried yeast which has retained excellent activity. The loss of activity, as seen in Compressed Yeast but in this case lost from the final ADY product, would appear to be a growth related phenomenon.

An interesting observation was made of the total solid determination for the activity assay. Dried yeast with no activity, produced a total solid residue which was dark brown in colour. Active Dried Yeast in contrast, produced a pale brown colour. This is illustrated in Fig. 3.23. This observation and reasons for the differences were not further pursued.

Fig. 3.21
1. A poor leavening yeast.
2. A moderate leavening yeast.
3. An excellent leavening yeast.

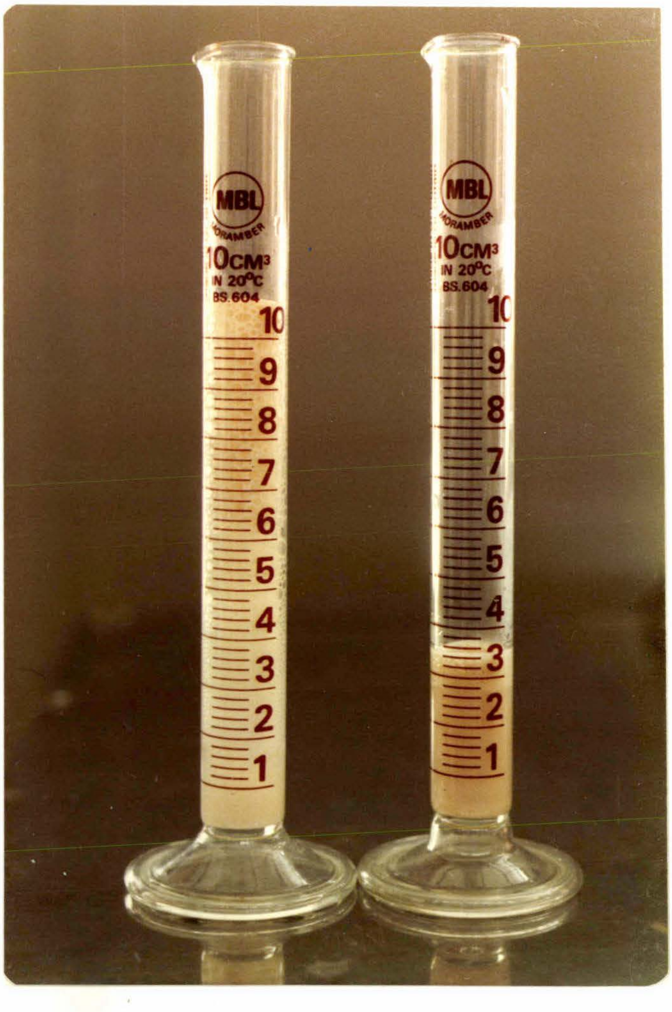


Fig. 3.22 Foaming Test
An active and non-active yeast

Fig. 3.23 Total solid residue from a non-active (left) and active (right) yeast.

4.0 DISCUSSION

4.1 Yeast Activity

Reed and Peppler (1973) state that ADY is produced using Saccharomyces cerevisiae as generally typified by strain ATCC 7752, sometimes called the 'dry yeast strain'. These present studies suggest that this description is misleading. The Compressed Yeast activity of ATCC 7752 was found to be lower than that of a commercial ADY preparation. Culture ATCC 7752 was obtained from the American Type Culture Collection in the freeze-dried state. In view of the lower activity noted with this strain it is possible that freeze-drying is not the best method of storing Baker's Yeast strains. Loss of viability has been associated with freeze drying (Atkin et al., 1962). Techniques used for organisms that do not survive lyophilization (frozen in 10% glycerol) may be more appropriate. More importantly, the ability of freeze-dried yeast to regain its leavening ability, even after successive subculturing has yet to be demonstrated. Mitchell and Enright (1957) reported that the viability of a culture may be a poor index to its leavening ability. This observation has been confirmed in the present study when ATCC 7752 was subcultured and so regained its full viability, although it was still not suitable as a stock dry yeast strain.

Two variables of Compressed Yeast storage conditions were investigated, viz. storage temperature and packaging material. During storage of Compressed Yeast at $4^{\circ}C$. there was no loss in activity over 3 weeks using waxed paper packaging. This was similar to Peppler's (1960) observations of a 10% loss in activity over a 4 week period at $4^{\circ}C$. Storage in the frozen state would appear to be a more practical approach. Little activity loss occurred during repeated freezing and thawing of Compressed Yeast, an observation also noted by Thiessen (1942).

Loss of activity occurs when storing Compressed Yeast, due to respiration and the associated metabolic heat production (Reed and Peppler, 1973). It was reasoned that this could be reduced by minimizing gas exchange and by cooling. However, the magnitude of the decline in activity when using polythene film suggested other factors were involved. Chester (1959) showed yeast cells could both respire and ferment their endogenous reserves; thus toxic fermentation products could have accumulated. Because activity losses were not observed using wax wrapped samples it might be postulated that the toxic metabolic products were lost by volatilization. Possible toxic products are ethanol or higher alcohols. Alternatively, but less likely, toxic compounds may have been leached from the polythene film. The advantage of reducing respiration through sealed packaging is counteracted by the loss of activity. Storage temperatures above 4^{o}C. accelerated this loss.

The amount of CO_2 evolved from fresh Compressed Yeast in a fermenting dough was determined. Using a modified Burrows and Harrison (1959) fermentometer, 17.0 mM CO_2/hr./g yeast solid was evolved. 18.8 mM CO_2/hr./g yeast solid was reported by Koch et al., (1954) using a straight (i.e. flour and water) dough. Investigations of liquid "pre-ferments" as used for continuous breadmaking (Garver et al., 1966) demonstrated that 23 mM CO_2/hr./g yeast solids were evolved at 31^{o}C. in 135 min. The present results show that New Zealand Compressed Yeast is of a similar quality to that produced overseas. The apparatus constructed also produced data comparable with published literature. With the Burrows and Harrison Fermentometer constructed from standard Quick-fit (Q F) glass-ware, there is little need for external standards (ADY) as suggested by Thorn and Reed (1959). Any future work must relate gas evolved to actual bake test data and that function presented in graphical or tabular form. Only then can the relative merits of differing gas measuring systems (Reed and Peppler, 1973) be ignored.

It is considered that the use of dough as substrate provides an experimental approach which resembles much more closely commercial practice than does the Warburg respirometric activity assay method. During the initial period of dough fermentation (a 13 min. interval), it was shown that endogenous respiration accounts for $^{2}/3$ of the gas evolved (section 3.1.3). Yeast samples with negligible leavening ability (section 3.3.2) still produced this initial gas. During this period enzyme systems would adapt to the anaerobic metabolism of flour sugars and the yeast cell's reserves be utilized. Warburg studies showed gas production was dependent on sugar type and concentration, as also reported by Shieh et al.,(1973). The difficulty in comparing these two quite different systems must be noted. Garver *et al*., (1966) stated that nutrients do influence the rate of gas evolution. In this study it was shown, not unexpectedly, that data obtained from Warburg studies can not be compared directly with the data derived from fermenting dough studies. Warburg conditions (e.g. sugar type and concentration) for CO_2 production measurements would require careful standardization if data comparison with dough tests are to be made.

There has been scant comment in literature concerning foam production during rehydration of ADY. As ADY rehydrates it forms a surface slurry which expands as CO_2 is evolved. As the same enzyme system that produces CO_2 from sucrose will also utilize maltose from flour, the foam produced is seen as a useful index of yeast activity. It is possible that such volumetric measurements of foam may provide the manufacturer with a rapid test of yeast activity.

The temperature of ADY rehydration is known to affect its activity (Peppler and Rudert, 1953; Herrera *et al*., 1956; Ponte *et al*., 1960). This work demonstrates that the nature of the rehydration solution also affects the dried yeast activity. Whether this has been appreciated in the past by different workers is not clear. There has been a vast array of solutions and solute concentrations used (see Table 1.2) but little comment on rationale or on their importance. These solutes increase the osmotic pressure of the rehydrating solution and thereby decrease leaching of cellular components. The particular solute involved (sucrose) would also contribute towards an early activation of enzyme systems later used in dough fermentation.

4.2 Drying Compressed Yeast

The relative humidity profile of ADY with respect to yeast activity was determined at $40^{o}C$., which is a common yeast drying temperature (Thorn and Reed, 1959). A clear optimum for activity was evident at 31% relative humidity. A humidity-activity relationship has been developed. Yeast activity did not increase at higher humidity values as reported by Shishatskii *et al*. (1971). As the relative humidity increases beyond 30%, the drying time also increases and, as expected, the activity declined.

The curvature of the humidity versus activity graph increased as the moisture content of the yeast decreased. Humidity control is therefore important in the latter stages of drying and is consistent with drying theory. During the constant rate period, moisture from within the cake is being continually supplied to the surface (Keey, 1978). However, during the falling rate period the yeast cake surface, consisting of yeast cells and possibly the cell walls of lysed cells, is drying. Cell death is presumed to be occurring. At this time the air humidity will be of greater importance for it is from the air that the balance of moisture at the surface must be maintained to minimise cell inactivation and death through dehydration. Thus yeast activity was clearly related to the drying air humidity.

Yeast metabolic enzymes are located in the cell wall and plasmalemma (MacWilliam, 1970) and moist heat will facilitate the denaturation of cellular proteins (Pelczar et al., 1977). This occurred when yeast was dried at 45% Hr and together with the long drying time was responsible for the decline in yeast activity. The drying rate was also related to the final ADY activity. At the two lower humidity values, 31% and 17% Hr, there was little difference in drying time. The highest drying rate however was not the most efficient for retaining yeast activity. An optimum drying rate (constant rate period) was determined at 31% Hr. At low humidity values the constant rate period was shortest and the cell surface will be dry for the longest period of time. Thus final ADY activity is a consequence of a compromise between drying time and drying rate, both controlled by the air humidity.

The drying time was within an acceptable period of 4 hrs. comparing well with the 3-6 hrs. of Thorn and Reed (1959) and 4.5 hrs. of Trevelyan (1973). The relationship between dried yeast activity, relative humidity and final moisture content was quantified for this particular drier. The basic drying parameters and mechanisms calculated (see section 3.2.1) will serve as a basis for further investigations of ADY drying characteristics.

It might be proposed that a similar relationship may be found for other drying methods. Although fluidized drying uses high initial temperatures and a high constant drying rate, Taylor (1975) recommends a 35% Hr value or less during drying. This Hr value is close to the optimum established in the current studies but Taylor's use of a high constant drying rate cannot be recommended.

The influence that the surface active agent glyceryl monostearate had on drying time was determined. Glyceryl monostearate was originally added as an aid to rehydration to reduce the surface tension of the rehydrating liquid (Chen et al., 1966). An emulsifier reduces the interfacial tension between a solid and liquid phase (Garrett, 1972). In the yeast strands being dried there would be an increased wetted surface area when an emulsifier was included. As capillary action was evident in the falling rate period, an increased wetted surface area would aid drying. The presence of an emulsifier also prevented case hardening of the extruded yeast strands. When case hardening occurs a hard impermeable surface skin forms which reduces the drying rate (Brennan et al., 1969). Clearly, the emulsifier reduced the drying time and hence the loss in activity. The inclusion of an antioxidant during drying did not however influence ADY activity. The protective nature of antioxidants is evident at moisture contents below 7.5% and during storage (Chen et al., 1966; Reed and Peppler, 1973). As a final moisture content of 9% was used and no storage trials undertaken, antioxidants were of no benefit in this study.

The sorption behaviour of dried yeast is important in two areas. Firstly, in process design where the case of water removal must be considered and secondly, in establishing the maximum amount of remaining moisture permissable for retaining dried yeast stability. Storage stability is an important ADY parameter. Very low moisture contents of ADY have been reported e.g. 2.2% ($^{w}/w$) by Mitchell and Enright, (1957). However, from the plotted isotherm (section 3.2.5) it would seem that even at this low moisture content Mitchell and Enright did not remove the water monolayer from their samples. Hence their yeast still retained it's activity upon rehydration. While the calculated moisture content for maximal yeast stability is lower than that used in commercial practice, the extra energy requirements needed to reduce the water from 8% to 2% and then to rehydrate the yeast must be considered.

The difference between the inflexion point of the ADY isotherm and the calculated monolayer (2.2% moisture) can be related to the various assumptions upon which the B E T model is based. A monolayer moisture content of 2.2% would classify it as group 3 of Salwins (1963) classification of B E T isotherms. Products in this group include potato flakes (5%) and spray-dried whole milk (3%) (Karel, 1975). Drying conditions used to produce these products may be applied to ADY production, and vice versa.

There was a correlation between the drying air and storage humidity conditions. The drying humidity optimal for dried yeast activity was 31% Hr at $40^{o}C$. while the equilibrium relative humidity for stored yeast at 8-9% moisture content was 33% Hr at $20^{o}C$. ADY dried below 8-9% moisture would require a package humidity below 33% to prevent partial rehydration.

Freeze-drying has received considerable attention as a preservation technique for micro-organisms (Wynants, 1962; Mazur, 1970; Heckly, 1978). Interest has, however, centred on the viability of the reconstituted micro-organisms (Atkin et al., 1962) rather than the subsequent fermentative aspects. Heckly (1978) stated that the freezing rate was not a critical factor for survival. In this work it was further shown that freezing rate was not critical for the retention of the fermentative characteristics of yeast metabolism. A freezing rate of $0.6^{o}C$./min. was found quite satisfactory while Mazur (1970) has reported a rate of $7^{o}C$./min, confirming Heckly's statement. Cryoinjury has been associated with the cell membrane (Mazur, 1970) as has the location of fermentative activity (McMurrough and Rose, 1967). It is likely that cryoinjury occurred in this study as there was a loss in leavening ability of freeze-dried yeast during the 2nd and 3rd hour. As the frozen yeast was exposed to room temperature during drying, water recrystalization may have occurred and a combination of freeze drying and vacuum drying took place. These conditions would allow cryoinjury and the subsequent loss in activity.

Acetone extraction was also considered. Trevelyan (1966), using dehydrating solvents, reported the difficulty in extracting lipids from whole yeast cells due to the low porosity of the cell wall. The complete loss of yeast activity in the current study suggests that the disruption must be severe.

4.3 Fermentation of Baker's Yeast

The yeast activity, or leavening ability of Compressed Yeast was correlated with five fermentation variables. Although the optimum fermentation parameters (carbon concentration, temperature, agitation) were identical for cell yield and yeast activity, they were significant at different values (e.g. high or low). The observed poor leavening response observed in some trials may have been an inability of yeasts to use the maltose from flour. However, the cause can be related to the fermentation conditions and in turn to the yeast's own metabolism and its regulation. The three significant parameters, listed above, will now be considered.

First, the role of glucose in fermentation is considered. The greatest leavening response occurred with yeast grown at low glucose concentrations. High glucose concentrations (5% $^w/w$) are known to induce catabolite repression (Phaff et al., 1978). Catabolite repression suppresses the Tricarboxylic Acid Cycle activity (viz. Isocitrate dehydrogenase (NAD^+)) and 6-Phosphofructokinase of the glycolysis pathway (Sols, 1971). Therefore low glucose concentrations avoid the repression of respiratory enzyme synthesis in the yeast. By design, low concentrations are maintained during commercial fermentations for maximum cell growth. This is achieved using incremental feeding (Reid, 1969). It is fortunate that these conditions also promote the development of Compressed Yeast with excellent leavening ability.

In contrast to the observations on cell activity, cell yield was maximal at high glucose concentrations. During laboratory fermentation this occurred as glucose was completely utilized even under anaerobic conditions. The duration of the fermentation (24 hrs.) resulted in the glucose concentration becoming limiting. Highest cell yields (4.67 mg/ml) therefore occurred with the highest glucose concentrations used in this study.

Secondly, oxygen also has a regulatory role in cell metabolism (Markhan, 1969). The direct role of oxygen is well known from the first work of Pasteur (Pasteur, 1876). It is also known that yeasts utilize glucose much more efficiently under aerobic conditions compared with anaerobic conditions (Barnett, 1976). The high substrate conditions used in this study (3% $^w/w$ and aerobic conditions) resulted in a high cell yield.

However, maximal activity occurred with yeast grown under anaerobic (or approaching anaerobic) conditions. It is unlikely that strict anaerobic conditions occurred and some oxygen diffusion did occur within the standing broth culture. Consequently, the TCA cycle would be operating to a limited extent. As dough conditions in the activity test are likely to be anaerobic rather than aerobic (Reed and Peppler, 1973), the observed activity may be related to the operation of a fully adapted anaerobic enzyme system.

In this study yeast biomass increased during the aerobic metabolism of glucose. It is presumed, that at the expense of CO_2 evolution, TCA intermediates initiated further cell biosynthesis. This was evident by the maximal cell yields observed. Chen (1959) stated 51% of CO_2 evolved during aerobic metabolism is via the decarboxylation of pyruvate, compared with 43% via the TCA oxidative cycle. It is postulated here that an adapted anaerobic system may produce in a dough a greater volume of CO_2 per glucose utilized than would an aerobic system. Chen and Peppler (1956) established a parallelism between pyruvate decarboxylation activity and anaerobic fermentation, as well as baking performance of yeast samples during drying. This would explain the greater activity observed with yeast grown under anaerobic conditions.

The third parameter considered was the fermentation temperature. Observed cell yields and yeast activities were highest at 28°C. Merritt (1966) reported that for Baker's Yeast the optimum cell yield occurred at approximately 30°C. and the maximum growth rate at 37°C. It is reasoned in this study that the optimum temperature for CO_2 evolution is related to the presence of viable and non-viable cells. Yeast activity was calculated on a "per gram dry weight basis", and not "viable cells". A decrease in viable cells would also be apparent as a decrease in the calculated activity. Stokes (1971) reported a decrease in viable cells at higher temperatures (40°C.). In this study it is likely that the ratio of viable to non-viable cells would be lower at 37°C. than at 28°C. in the fermentation broths. With fewer viable cells present in the dough when the yeast is grown at 37°C., less CO_2 would be evolved.

Further to this, all sugar was utilized during fermentation. Hough and Maddox (1970) stated that cell autolysis occurs only after substrate exhaustion. Thus substrate exhaustion would add to the increase in non-viable cells at 37°C. (the growth temperature used), by accelerating the occurrence of autolysis.

The principle fermentation parameters as currently established for maximizing cell yields under conventional Baker's Yeast production conditions were compared with those significant parameters which allowed the development of yeast with maximum leavening ability. Low sugar concentrations (0.5%) and temperature values (30°C.) were common to both fermentations. However, maximum cell yield required aerobic conditions whereas maximal leavening ability required anaerobic conditions. It was likely that interactions between fermentation parameters occurred, for example between glucose concentrations and dissolved oxygen level. Further studies are required to optimize the dissolved oxygen concentration during fermentation in terms of cell yield and yeast leavening ability.

The yeast response to the three fermentation parameters considered previously also altered the final broth pH. However, the change in pH did not significantly affect the Compressed Yeast's activity. It has been reported (White, 1954) that the internal pH of a yeast remains constant despite outside variations. This is one reason why the initial broth pH did not appear to affect yeast activity. Also, observations of pH (Garver et al., 1966) during continuous breadmaking suggested that pH played a greater role in dough structure than did CO_2 evolution. This study confirms that in broth cultures pH does not play a significant role with respect to yeast activity. As gas production was monitored, other roles of pH were not recorded. In terms of yeast activity the fermentation pH can be optimized for other variables (e.g. to minimise contamination or maximise CO_2 retention in dough) without producing a poor leavening yeast.

The investigation of activity loss during the drying of laboratory grown yeast was less successful. As yeast multiplied, over several generations, their ability to survive drying declined. Various reasons for this were considered. After several tests with various yeast starter cultures, cultural factors relating to the S. cerevisiae were eliminated. However, it was not possible to screen all environmental factors that are likely to affect yeast activity and the reason for activity loss is as yet undetermined.

Two further observations associated with fermentation studies warrant comment; these being the colour of ADY and total solids residue. Colonies of most yeast species appeared white with a slight greyish or yellowish shade. Drying intensified any colouring present in the moist yeast. Pigment production, as such, is not normally associated with Saccharomyces cerevisiae (Phaff et al., 1978). Any observed influences that environmental parameters (growth temperature, dissolved oxygen and initial pH) have on components of colour (hue, value and chroma) may be related to an oxidative reaction occurring, Maillard browning reactions, the accumulation of particular cell components, or the presence of antioxidant and emulsifier agents.

The light colour residue from an active yeast in solution was interesting. Inactive yeast resulted in a dark brown residue. It is possible that this phenomenon was related to the presence or absence of active enzymes in the yeast cell. The enzymes in inactive yeast may be able to interact with other cell components or compounds in solution. As mentioned previously this was not pursued further.

It was regrettable that laboratory-prepared yeast with maximal leavening ability was unable to maintain its activity during drying, under what had been established as optimal drying conditions. Future investigation should bring these two optimized processes together. When this is achieved, it will be possible to provide an ADY superior in activity to that which is presently available.

5.0 CONCLUSIONS

1. A manometric technique for the laboratory measurement of yeast leavening ability using flour as a substrate was found convenient, reproducible and provided a system which closely resembled industrial practice. An alternative respirometric procedure involving Warburg studies with liquid substrates produced data that was difficult to relate to rate curve data obtained with flour substrates. Foam production during rehydration was used as an indication of the potential leavening ability of ADY.

2. Results from the activity assay were influenced by the rehydration solution employed, the particular yeast strain and the occurrence of endogenous respiration.

3. Humidity control during the falling rate period of drying, 31% Hr @ $40^{o}C.$, minimised activity losses. Final ADY activity can be determined as a function of air humidity, time and emulsifier concentration from a developed empirical model. An example of such a model is presented.

$$Ac = 11.53 - 2.33\ Hr + 1.87\ W_F - 2.867(Hr)^2$$

where Ac = dried yeast activity ($mMCO_2$/hr/g yeast solid)
Hr = relative humidity at $40^{o}C$.
W_F = final moisture content.

As ADY activity is related to air humidity, the optimum drying rate for retaining activity was not the maximal observed. Emulsifying agents sharply reduce the drying time. The concentration of emulsifier for optimum drying was not determined in these studies.

4. During freeze-drying, the freezing rate did not affect the yeasts fermentative character. However, the yeast activity was not retained in this study of freeze-drying. Careful control of sublimation conditions will be required to maintain the yeasts activity.

5. The leavening ability of Compressed Yeast was influenced by three

fermentation variables, viz. the sugar concentration, agitation rate/dissolved oxygen concentration and growth temperature. Conditions relating to growth temperature and sugar concentration were similar for both optimal cell yield and yeast leavening ability. However, maximal activity occurred when yeasts were grown under anaerobic conditions. In contrast, greatest cell yields occurred under agitated conditions. However, the optimum dissolved oxygen concentration needed during the fermentation for best cell yield and yeast activity requires further study.

It was found that during the fermentation neither the initial broth pH (and change in this pH during growth) nor the carbon to nitrogen ratio of the fermentation broth affected significantly the yeast's leavening ability.

6. Further developments in ADY production are likely to follow when the optimal conditions for the two processes studied are combined, namely, Compressed Yeast fermentation and drying of Compressed Baker's Yeast.

APPENDIX 1. YEAST ACTIVITY CALCULATIONS

1. Analysis of Salt Suspending Solution

Burrows and Harrison, (1959) used 3.57 g Compressed Yeast @ 31.25% total solids, per 100 mls. of salt solution in their apparatus.

The composition of the yeast - salt solution added to flour was as listed below

		Total solids (g/100 mls)
Yeast - salt solution		= 3.78
Yeast solids	3.57 x 0.3125	= 1.1156
Remaining solids	$(NH_4)_2SO_4$ NaCl	= 2.7

2. Amount of Sample to be Assayed.

for example

Moisture content is approximately 10%

Total yeast solids (T.S.) = 90%

then $\chi \times (T.S.) = 1.1156$

$$\chi = \frac{1.1156}{0.9}$$

Amount of sample 1.2396 g yeast per 100 mls salt solution

= 0.3100 g yeast per 25 mls salt solution

3. Conversion of mls. CO_2 evolved to millimole CO_2/hr./g yeast solid.

Using the observed data in Table A.1.1 the yeast activity is calculated in the steps below.

a) Initial conversion to 20°C. and 760 mm Hg.

$$\text{As } \frac{P_1V_1}{t_1} = \frac{P_2V_2}{t_2}$$

$$\text{then } V_2 = \frac{P_1V_1t_2}{t_1P_2}$$

$$\Rightarrow V_2 = \frac{1000 \times 31.6 \times 293}{(273 + 20.5) \times 1013}$$

$= 31.14$ mls CO_2 evolved at 20^{o}C. and 760 mm Hg.

b) To standard yeast solids

$$\Longrightarrow \quad \frac{3.78}{3.84} \quad \text{multiplied by } 31.14$$

$= 30.65$ mls CO_2 evolved at 20^{o}C. and 760 mm Hg at std. total solids.

c) To S.T.P.

$$\Longrightarrow \quad \frac{273}{293} \quad \text{multiplied by } 30.65$$

$= 28.56$ mls CO_2 evolved at S.T.P.

d) To mls per hour

Total time of dough fermentation is 13 + 45 + 45 = 103 min.

$$\Longrightarrow \quad \frac{28.56}{103} \times 60$$

$= 16.64$ mls CO_2 at S.T.P. per hour

e) To millimoles CO_2/hr

As $22.262\ \ell\ CO_2 = 1$ mole CO_2 at S.T.P.

$$\frac{16.64}{22.262}$$

$= 0.7474$ millimole CO_2 per hour

f) To gram basis

Yeast solids $= 1.1156$ g/100 mls

$= 0.2789$ g/25 mls

$= 0.0446$ g/4 mls

$\Longrightarrow$ 0.7474 millimole CO_2/hr/0.0446 g

$=$ 16.76 millimole CO_2/hr./g yeast solids

g) Error present

The major source of error in the yeast activity term is the measurement of gas evolved; $\pm$ 0.25 mls, a range of 0.5 ml.

$= 16.8 \pm 0.1$ millimole CO_2/hr./g yeast solid.

Table A.1.1 Observed Data for a Yeast Activity Assay

	Symbol	units	Value
Average gas evolved	V_1	mls gas	31.6 ± 0.25
Room temperature	t_1	$^{o}C.$	20.5 ± 0.05
Atmospheric pressure	P_1	Pa	1000.0 ± 1.0
Total solids	T.S.	g.	3.84 ± 0.005

Fig. A1.1 Drying Curve for Extruded Yeast Strands Using an Infra-red Balance

Sample Moisture Content after 15 mins. = 316.0 % (dry weight)

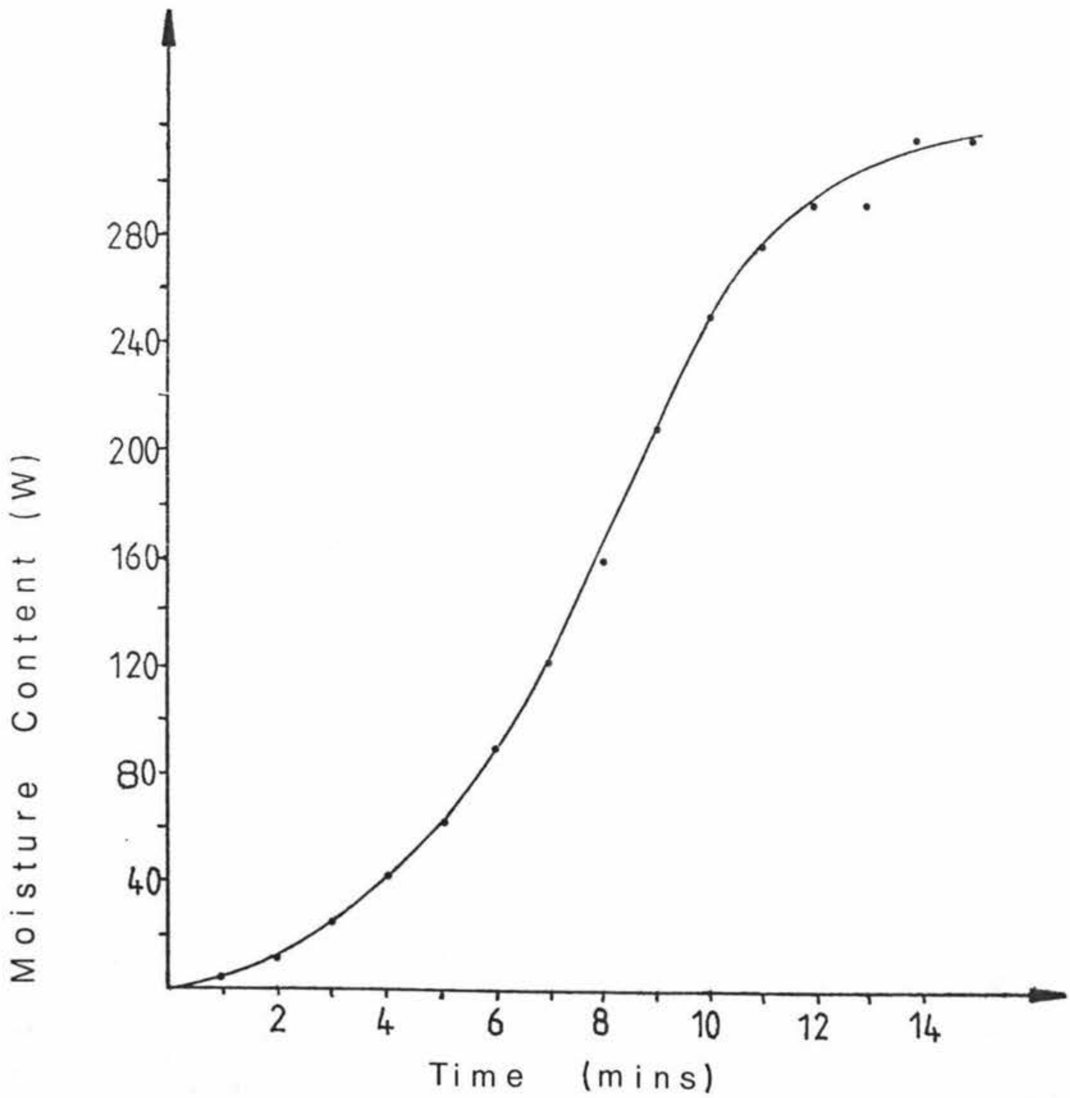


Fig. A2.1 Activity Decline vs Time for Compressed Yeast Stored at $4\,^{\circ}C$

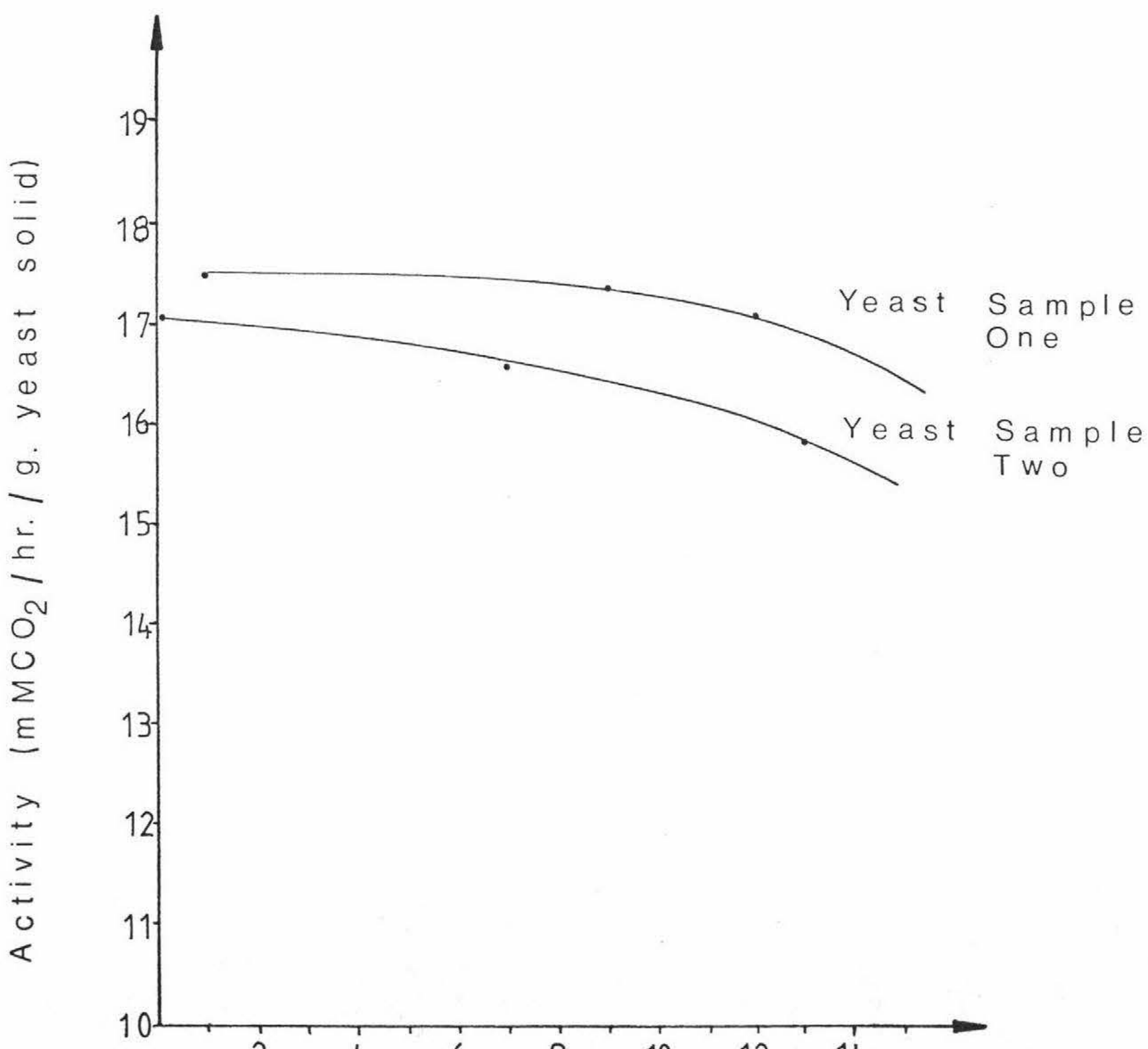
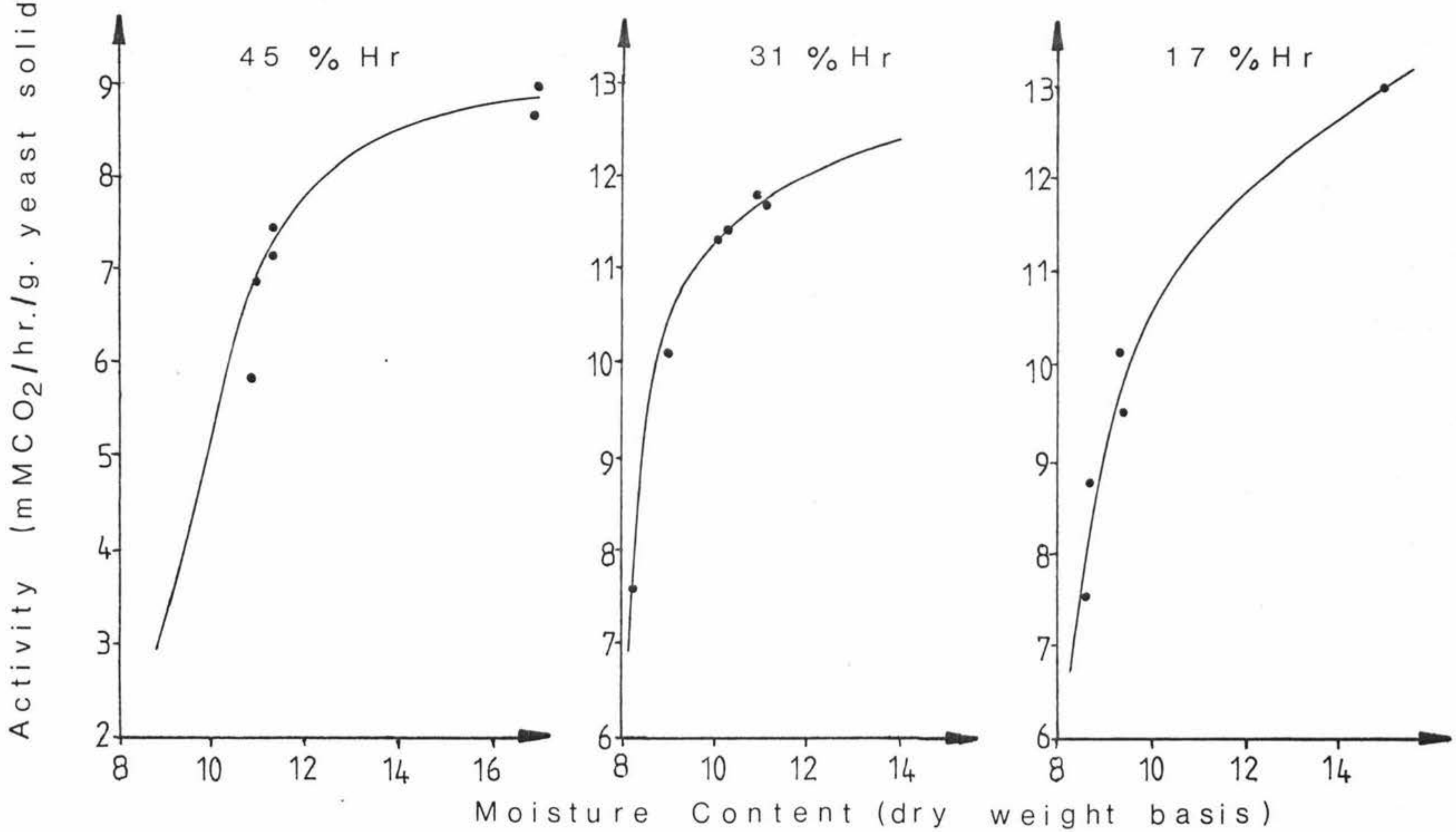


Table A.2.1 Activity Correction Factor Obtained from Fig. A.2.1 for Compressed Yeast

Run No.	day No.	activity via graph (1)	correction factor
5 yeast 1	10	17.31	1.00
6	11	17.24	0.996
7	12	17.11	0.989
8 yeast 2	0	17.11	0.989
9	1	17.05	0.985
10	2	17.00	0.980
11	8	16.57	0.957
12	9	16.47	0.952
13	9	16.47	0.952
14	10	16.36	0.945
15	10	16.36	0.945

(1) activity expressed as $mMCO_2$/hr./g yeast solid.

Fig. A2.2 Standard Curves for Activity of Active Dry Yeast versus Moisture Content



APPENDIX 3. DRYING RATE CALCULATIONS

Sample calculation for drying rate at 31% H_r at $40^{o}C$.

1. Tray dimensions

$$0.28m \times 0.27m$$

$$\text{Surface area} = 0.28 \times 0.27 \times 2$$
$$= 0.151\ m^2$$

2. Area yeast strand

$$2 \times \pi \times \text{dia. strand} \times \text{length strand}$$
$$2 \times \pi \times 0.0015m \times 0.23m$$
$$= 0.0022\ m^2 / \text{strand}$$
$$= 0.033\ m^2 / 15 \text{ strands} / \text{tray}$$

3. Effective surface area

$$\frac{m^2 \text{ product}}{m^2 \text{ tray}}$$

$$= \frac{0.033}{0.151}$$

$$= 0.218\ m^2$$

4. Drying rate *

$$\frac{dW}{dt} \times \frac{1}{\text{Area}} \times \frac{1}{60}$$

$$= 4.7 \times 10^{-3}\ kg/\ /m^2 \text{ (kg. dry material)}$$

* see table A.3.1.

5. Surface heat transfer coefficient, hc from constant rate period

$$\text{Rate} = 4.3 \times 10^{-3}\ kg/s\ m^2 \text{ (kg dry solid)}$$

$$\text{Rate} = \frac{hc\ (td - tw)}{h_{fg}}$$

where $h_{fg} = 2441 \times 10^3$ J/kg

humidity = 31%

dry bulb temp. = $40^{o}C$.

wet bulb temp. = $25.3^{o}C$.

$$hc = \frac{4.3 \times 10^{-3} \times 2441 \times 10^3}{14.7}$$

$$= 0.714\ Kw/m^2\ {}^{o}C. \text{ (kg. dry solid)}$$

kg dry material

kg dry material.

100 g @ 30% solids → x g @ 92% solids → 32.6 g

→ hc = 23.3 $W/m^2\ ^oC$.

Table A.3.1 Datq and Calculations for Drying Rate Determination at 31% H_r at 40^oC.

t (hr)	W $\left(\frac{g\ H_2O}{g\ yeast\ solid}\right)$	$\frac{dW}{dt}$	Rate $\times 10^{-3}$ ($kg/S/m^2$ kg dry product)
0.375	1.60		4.7
0.5	1.26		4.1
0.625	0.93		3.95
0.75	0.68		2.25
0.875	0.50		1.48
1	0.40		1.1
1.125	0.30		0.79
1.25	0.25		0.61
1.375	0.20		0.44
1.5	0.18		0.34
1.75	0.14		0.12
2	0.12		0.069
4	0.09		

APPENDIX 4. EXPERIMENTAL DESIGN

Table A.4.1 The 2_{IV}^{8-4} Fold Over Design (Box and Hunter, 1961) for Fermentation Conditions

Run no.	A	B	C	D	E	F	G	H	
1	-1	-1	-1	-1	-1	-1	-1	-1	(1)
2	1	-1	-1	-1	1	1	-1	1	
3	-1	1	-1	-1	1	-1	1	1	Principal fraction
4	1	1	-1	-1	-1	1	1	-1	2_{III}^{7-4}
5	-1	-1	1	-1	-1	1	1	1	
6	1	-1	1	-1	1	-1	1	-1	
7	-1	1	1	-1	1	1	-1	-1	
8	1	1	1	-1	-1	-1	-1	1	
9	-1	-1	-1	1	1	1	1	-1	
10	1	-1	-1	1	-1	-1	1	1	Principal fraction
11	-1	1	-1	1	-1	1	-1	1	with all signs
12	1	1	-1	1	1	-1	-1	-1	reversed
13	-1	-1	1	1	1	-1	-1	1	
14	1	-1	1	1	-1	1	-1	-1	
15	-1	1	1	1	-1	-1	1	-1	
16	1	1	1	1	1	1	1	1	

(1) In conjunction with Table 2.9 ; Run 1 fermentation conditions would read 28°C., pH 4, 0.3% N, Dummy factor, 0.5% C. not agitated, Dummy factor, Dummy factor.

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