YEAST METABOLISM IN FRESH AND FROZEN DOUGH

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ABSTRACT

Fresh bakery products have a very short shelf life, which limits the extent to which manufacturing can be centralised. Frozen doughs are relatively stable and can be manufactured in large volumes, distributed and baked on-demand at the point of sale or consumption. With appropriate formulation and processing a shelf life of several months can be achieved.

Shelf life is limited by a decline in proofing rate after thawing, which is attributed to a) the dough losing its ability to retain gas and b) insufficient gas production, i.e. yeast activity. The loss of shelf life is accelerated by delays between mixing and freezing, which allow yeast cells the chance to ferment carbohydrates.

This work examined the reasons for insufficient gas production after thawing frozen dough and the effect of pre-freezing fermentation on shelf life. Literature data on yeast metabolite dynamics in fermenting dough were incomplete. In particular there were few data on the accumulation of ethanol, a major fermentation end product which can be injurious to yeast.

Doughs were prepared in a domestic breadmaker using compressed yeast from a local manufacturer and analysed for glucose, fructose, sucrose, maltose and ethanol. Gas production after thawing declined within 48 hours of frozen storage. This was accelerated by 30 or 90 minutes of fermentation at 30˚C prior to freezing.

Sucrose was rapidly hydrolysed and yeast consumed glucose in preference to fructose. Maltose was not consumed while other sugars remained. Ethanol, accumulated from consumption of glucose and fructose, was produced in approximately equal amounts to CO_2, indicating that yeast cells metabolised reductively.

Glucose uptake in fermenting dough followed simple hyperbolic kinetics and fructose uptake was competitively inhibited by glucose. Mathematical modelling indicated that diffusion of sugars and ethanol in dough occurred quickly enough to eliminate solute gradients brought about by yeast metabolism.

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LIST OF ABBREVIATIONS

DETA	dielectric thermal analysis	
DM	dry matter	
DMA	dynamic mechanical analysis	
DMTA	dynamic mechanical thermal analysis	
DSC	differential scanning calorimetry	
DTA	differential thermal analysis	
NMR	nuclear magnetic resonance	
SD	standard deviation	
SE	standard error	
TAM	total available monosaccharides	[mmol.(100g dough)$^{-1}$]

LIST OF SYMBOLS USED

a_w	water activity	-
A	area	[m^2]
A_{dough}	area of dough per yeast cell	[mm^2]
c	solute concentration	[mol.L^{-1}]
D	diffusion coefficient	[m^2.s^{-1}]
D_e	estimated diffusion coefficient for ethanol diffusing in dough aqueous phase	[cm^2.s^{-1}]
D_g	estimated diffusion coefficient for glucose diffusing in dough aqueous phase	[cm^2.s^{-1}]
E	ethanol concentration	[mol.L^{-1}]

E_i	initial ethanol concentration	[mol.L^{-1}]
E_{max}	maximum ethanol concentration at which sugar uptake occurs	[mol.L^{-1}]
F_i	initial fructose concentration	[mol.L^{-1}]
G_i	initial glucose concentration	[mol.L^{-1}]
G_R	glucose consumption rate (microsystem model)	[mol.L^{-1}.min^{-1}]
i	space partition point number	-
j	time partition point number	-
J	diffusion flux	[mol.m^{-2}.s^{-1}]
$J(x)$	diffusion flux at position x	[mol.m^{-2}.s^{-1}]
k	Boltzmann constant	[N.m.K^{-1}]
K_i	competitive inhibition constant	[mol.L^{-1}]
K_m	affinity constant	[mmol.L^{-1}]
L	radius of the sphere of aqueous phase in the microsystem model	[m]
M	molar mass	[g.mol^{-1}]
n	number of samples or replicates	-
Q_{st}	net isoteric heat of adsorption	[kJ.mol^{-1}]
r	surface radius of curvature	[m]
r_{dough}	radius of the hypothetical circle of dough allocated to each yeast cell	[mm]
R	universal gas constant	[N.m.K^{-1}.mol^{-1}]
S	sugar concentration	[mmol.L^{-1}]
S_A	concentration of substrate A	[mmol.L^{-1}]
S_I	concentration of inhibitor	[mmol.L^{-1}]
t	time	[min]

t_n	time co-ordinate number ‘n’	-
ΔT_f	freezing point depression	[˚C]
T	absolute temperature	[K]
V	rate of sugar uptake	[mmol.L^{-1}.h^{-1}]
V_m	mean volume	[ml]
V_M	molar volume	[m^3.mol^{-1}]
V_{max}	maximum specific rate of sugar uptake	[mmol.(g biomass)$^{-1}$.h^{-1}]
V_G	glucose uptake rate	[mmol.(100g dough)$^{-1}$.min^{-1}]
V_F	fructose uptake rate	[mmol.(100g dough)$^{-1}$.min^{-1}]
x_n	space co-ordinate number ‘n’	-
X	biomass concentration	[g.L^{-1}]
Y_{ef}	molar yield of ethanol from fructose	-
Y_{eg}	molar yield of ethanol from glucose	-

GREEK SYMBOLS

α	ethanol inhibition coefficient	-
γ	surface tension	[N.m^{-1}]
η	coefficient of viscosity	[N.s.m^{-2}]
π	3.14159	-
ρ	density	[g.m^{-3}]

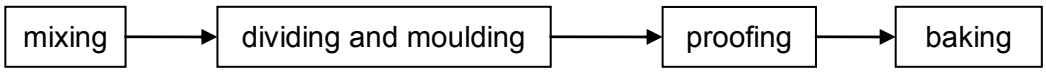
1. LITERATURE REVIEW

1.1. Breadmaking processes and formulations

Breadmaking processes which include yeast as the leavening agent share three basic requirements: (a) to develop a dough structure capable of retaining gas, (b) to introduce gas bubble nuclei into the dough and (c) to provide conditions for yeast cells to produce the gas that makes the dough rise (Collins 1985). There are four main breadmaking processes: 'straight dough', 'sponge and dough', 'mechanical dough development', and 'activated dough development.'

In traditional 'straight dough' or bulk fermentation processes dough structure is developed by several hours of proofing, which may include one or more 'knockdown' or remoulding stages (Figure 1.1).

Figure 1.1. Simplified straight dough process.



A basic straight dough formulation is given in Table 1.1, showing ingredients both as a percentage of total weight and on the basis of 100 parts flour, a common convention in industry.

Table 1.1. Straight dough formulation.

ingredient	*flour basis*	*% total*
flour	100	55.8
water	64	35.7
compressed yeast	2.5	1.4
salt	2	1.1
sugar	8	4.5
shortening	2.75	1.5

Reproduced from Kulp and Ponte (2000).

'Mechanical dough development' uses high-speed mixing and chemical dough improvers to develop dough structure, which eliminates the need for prolonged fermentation (Collins 1985; Myers et al. 1998). In 'sponge and dough' processes, part

of the flour and water is combined with yeast in a ‘sponge’, which is fermented for 3-5 hours before remaining ingredients are mixed in (Pyler 1988; Kulp and Ponte 2000). ‘Activated dough development’ employs reducing agents such as cysteine and sodium metabisulphite in combination with oxidising improvers and added fats (Collins 1985).

Dry ingredients are hydrated and formed into an homogeneous mass during mixing. A structure capable of holding gas is formed and air is entrained into the dough (Collins 1985; Pyler 1988). Structure arises primarily from the interaction of wheat proteins via noncovalent hydrogen bonds, covalent disulphide links, hydrophobic interactions and chain entanglement (Bloksma 1971; Potus et al. 1992). The mechanical force required to mix a dough at constant speed typically rises to a peak at optimum dough development, then declines with over-mixing.

Dough is usually rested for a short time after mixing then divided into pieces of the desired weight and moulded into shape. Moulding redistributes gas cells evenly and further develops dough structure (Pyler 1988).

During proofing yeast cells consume fermentable sugars and excrete carbon dioxide and ethanol. Carbon dioxide dissolves in dough and evaporates into the air bubbles introduced by mixing, expanding the dough (Giannou et al. 2003).

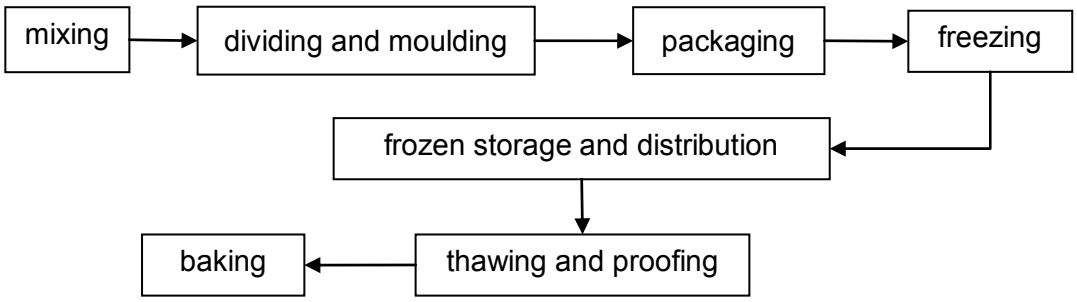
The solubility of CO_2 falls as temperature rises during baking and dissolved CO_2 evaporates into gas cells, producing a rapid expansion of dough known as ‘oven spring’. It is often stated that oven spring is due to accelerated yeast activity with increasing temperature, but the yeast’s contribution to oven spring is small (Dennis Lonergan, pers. comm. 2005). Other changes in baking include denaturation of protein, gelatinisation of starch, crust formation and browning reactions (Ponte 1973; Sablani et al. 2002).

1.2. Frozen dough process

Freshly-baked products are highly perishable and rapidly lose organoleptic appeal due to staling and growth of microorganisms (Stauffer 1993). This limits the possibility of centralised production due to the demands placed on the transport chain (Räsänen 1998).

Frozen bakery products are more amenable to distribution. There are three main types of frozen bakery products: 'bake-off' frozen doughs, 'par-baked' and fully-baked products (Best 1995). 'Bake-off' frozen doughs are doughs which have been mixed, divided and moulded then frozen. They must be thawed, proofed and baked before consumption (Figure 1.2). The straight dough process is most commonly employed for producing frozen doughs (Kulp 1995), but the sponge and dough method has also been successfully used (Sugihara and Kline 1968).

Figure 1.2. 'Bake-off' frozen dough process.



'Par-baked' products have been proofed and partially baked prior to freezing, and require thawing and a short final baking. Fully-baked products are simply reheated prior to consumption.

A variety of freezing technologies are available, including air-blast, contact and cryogenic (George 1997). Frozen dough packaging must perform functions such as contain, protect and identify the product, and moisture barrier properties are important due to the dryness of air in cold storage facilities (Lorenz and Kulp 1995; Varriano-Marston 1995).

Frozen doughs are typically thawed at 1 to 4˚C for 3-18 hours prior to final proofing at 32 to 43˚C (Lorenz and Kulp 1995). Thawing and proofing may be carried out at

constant temperature or with progressively increasing temperature to avoid surface condensation and uneven proofing (Lorenz and Kulp 1995).

Unfermented frozen doughs must retain the ability to proof after thawing, since they are not proofed before freezing. Frozen shelf life is limited by an increase in the time taken for the thawed dough to proof to a desirable volume before being baked (Bruinsma and Giesenschlag 1984; Inoue and Bushuk 1991). Pre-fermenting doughs before freezing reduces the requirement for post-thaw proofing, but prefermented doughs are more susceptible to structural damage from ice crystals than are unfermented doughs (Räsänen 1998).

Proofing power is affected mainly by yeast activity (CO_2 production) and gas retention, which is a function of dough rheology (Casey and Foy 1995). High-protein flours and increased levels of dough conditioners are recommended to achieve adequate gas retention (Collins 1985; Inoue and Bushuk 1992; Lorenz and Kulp 1995). More yeast is needed to compensate for loss of activity during frozen storage (Collins 1985; Lorenz and Kulp 1995).

The treatment of dough prior to the freezing process has a profound impact on the frozen shelf life. For example, delayed addition of yeast and salt can reduce the loss of yeast activity (Dubois and Blockolsky 1986; Neyreneuf and Van der Plaat 1991). Particularly notable is the detrimental effect of fermentation prior to freezing ('pre-fermentation') on shelf life (Casey and Foy 1995).

The reasons for this have remained elusive, and form the basis of the current project.

1.3. Raw Materials and their roles in breadmaking

The most important ingredients of bread are wheat flour, water, salt, fats, sugars, small quantities of chemical 'improvers' or 'conditioners' and yeast.

1.3.1 Flour

The baking performance of wheat flour is far superior to other cereals, but composite flours containing rye or other cereals are sometimes used in breadmaking (Eliasson and Larsson 1993). Flour consists of proteins, carbohydrates, lipids, moisture, vitamins and minerals.

Wheat flour contains 6-20% protein (MacRitchie 1984; Eliasson and Larsson 1993; Lookhart and Bean 2000) and flours suitable for breadmaking contain 11-13% protein (Kulp and Ponte 2000). Wheat flours suitable for breadmaking are referred to as 'hard' whereas 'soft' flours are more suitable for cakes and other bakery products in which wheat protein is not the most important structural component. The terms 'hard' and 'soft' were originally an indication of the force required to mill wheat, and it is now recognised that this is not strictly correlated to protein content; quantitatively minor wheat components can significantly impact on wheat hardness.

The main proteins of wheat are the glutenins and gliadins. Glutenins make up 40-50% of flour protein and are capable of both intra- and inter-molecular disulphide bonding via cysteine residues, which gives them the ability to form large polymers in dough (Eliasson and Larsson 1993; Lasztity 1996; Weegels et al. 1996). This fraction contributes strength and elasticity, and is primarily responsible for the mixing characteristics of dough (MacRitchie 1984; Eliasson and Larsson 1993; Weegels et al. 1996).

Gliadins constitute 30-35% of total protein and form exclusively intramolecular disulphide bonds (Lindsay and Skerritt 1999). They contribute plasticity or extensibility to a dough (Schwartzberg and Hartel 1992). Gliadins appear to have a 'space-filling' role in dough structure (Shogren et al. 1969; Amend and Belitz 1990; Weegels et al. 1996; Lindsay and Skerritt J.H. 2000).

As proteins are wetted and sheared during mixing, glutenins and gliadins form a cohesive and visco-elastic water-insoluble complex known as gluten. Gluten can be stretched into thin films capable of retaining gas, which is why wheat doughs expand during proofing and yield a light, palatable loaf on baking (Giannou et al. 2003). The

remainder of flour proteins are soluble in water, and include enzymes such as amylases, which hydrolyse starch, and proteases, which may affect dough rheology (Eliasson and Larsson 1993).

Lipids make up 2-3% of flour, and reconstitution studies have found that loaf volume passes through a minimum at lipid content between that of defatted flours and whole flours (Macritchie and Gras 1973; MacRitchie 1978). Polar lipids improve loaf volume due to foam-stabilising effects (Macritchie 1977, 1981, 1984; Macritchie and Gras 1973. Correlations between the composition of lipid fraction and breadmaking potential of different flours have been found in some studies (Chung et al. 1980, 1982; Bekes et al. 1986; Morrison et al. 1989), but others reported little or no correlation (Marston and Macritchie 1985; Larsen et al. 1989; McCormack et al. 1991; Ernandes et al. 1992).

The main carbohydrates of wheat flour are starch, non-starch polysaccharides and sugars. Starch is the largest single component of wheat flour, typically comprising 75-90% of dry matter (Medcalf and Gilles 1968; Eliasson and Larsson 1993; Preston 1998). It is present in the form of granules consisting of the glucose polymers amylose and amylopectin.

Amylose contains almost exclusively α-1,4 linkages, and its linear chains forms crystalline regions within granules, whereas amylopectin contains many α-1,6 branch points and forms amorphous regions (Medcalf and Gilles 1968; Myhre 1970; Shelton and D'Appolonia 1985; Eliasson and Larsson 1993). Wheat starch granules have a bimodal size distribution consisting of lenticular A-starch granules with diameter 14-35 μm and spherical B-starch granules with diameter 2-10 μm (Sandstedt 1961; Medcalf and Gilles 1968; Myhre 1970; Shelton and D'Appolonia 1985; Eliasson and Larsson 1993).

In dough, starch granules may contribute to the stability of gas cell lamellae (Sandstedt et al. 1954; Sandstedt 1961) or act as inert ‘filler’ particles (Bloksma 1990b; Tolstoguzov 1997). Granules which are mechanically damaged in milling absorb water and become susceptible to attack by endogenous or exogenous hydrolytic enzymes, yielding fermentable carbohydrates (Sandstedt 1961; Hoseney et al. 1971; MacRitchie 1984; Shelton and D'Appolonia 1985; Preston 1998; Bushuk 1998).

Non-starch polysaccharides (NSP) include celluloses, polymers of pentose sugars (pentosans), polymers of glucose and fructose (glucofructans) and hydrophobic lignins (Table 1.2).

Table 1.2. Composition of non-starch polysaccharides found in wheat flour.

Component	*% wheat*	*% white flour*
total pentosans	5.2-7.0	1.13-3
water-soluble pentosans	0.68-0.70	0.43-1.5
cellulose	2-3	0.6
total beta glucans	0.7-4.0	<1.1
lignin	0.5-1.2	–
glucofructans	1.4	0.41-0.46

Compiled from D'Appolonia et al. (1971), Girhammar et al. (1988), Pomeranz (1988), Ugalde and Jenner (1990), Marion et al. (1998), and Shelton and Lee (2000).

The influence of pentosans on dough and bread properties is threefold: (a) water-insoluble pentosans swell and rapidly absorb substantial amounts of water, (b) water-soluble pentosans can undergo oxidative gelation with each other or with proteins to form water-holding gels and (c) pentosans contribute to the gas-holding properties of dough (Shelton and D'Appolonia 1985; Meuser and Suckow 1986; Izidorczyk et al. 1991; Wang et al. 2002; Roman-Gutierrez et al. 2002a). The gel structures formed between pentosans and proteins act as a water 'reservoir' in bread, slowing changes associated with staling (Meuser and Suckow 1986).

1.3.2 Water

Water is essential to properly disperse ingredients, dissolve sugars and salt and transform raw materials into a cohesive mass (Giannou et al. 2003). It acts as a lubricant (Webb et al. 1970) and gives rise to hydrogen-bonding and hydrophobic interactions within and between proteins (Bloksma 1990b; Potus et al. 1992).

Other functions include hydrating pentosans and damaged starch (Kulp and Ponte 2000), activating enzymes (Lee 1970) and facilitating interactions between dissolved and/or suspended dough components, e.g. yeast cells and sugars or enzymes and starch granules (MacRitchie 1976; Bloksma 1990b; Eliasson and Larsson 1993; Giannou et al. 2003).

The amount of water in a dough formulation depends on the composition of the flour and the process employed, but is generally in the region 55-70 grams per 100 grams of flour (Kulp and Ponte 2000).

1.3.3 Yeast

In this section, 'yeast' refers to *Saccharomyces cerevisiae* unless otherwise indicated. It is generally true that baker's yeast is *Saccharomyces cerevisiae*, but only specialised strains are suitable for baking (Rasper and Walker 2000).

Yeast serves three main functions in breadmaking: leavening dough, favourably modifying the rheological properties of dough and contributing flavour and aroma compounds (Reed and Nagodawithana 1991; Giannou et al. 2003). Baker's yeast is available in bulk liquid form (18-22% solids), in compressed cakes (28-33% solids) and in several dried forms (92-96% solids) (Chen and Chiger 1985).

Yeast cells are quite sensitive to osmotic pressure. The limiting water content for yeast activity in dough is around 30-35% (flour basis) and gassing power increases with increasing water content up to 100% (Lee 1970; Shogren et al. 1977). Water content of ~55-70% (flour basis) produces doughs with acceptable rheological properties, i.e. rheological optimum is somewhat less than the optimum for yeast cell metabolism. Salt suppresses yeast activity (Marek and Bushuk 1967; Chen and Chiger 1985) and high levels of sugar in dough will inhibit gas production (Schultz 1965; Marek and Bushuk 1967; Suomalainen et al. 1972; Saalfeld and Freund 1998).

1.3.4 Sugars

The sugars in dough arise from:

- sugars originally present in the flour
- sugars added as ingredients
- sugars enzymatically cleaved from oligosaccharides or polysaccharides

Flour milled from sound wheat contains low levels of free mono- and di-saccharides (Table 1.3). If wheat has been improperly stored it will germinate. This will result in increased α-amylase activity and starch will be hydrolysed, producing short glucose polymers and free glucose (Bock 2000).

The amount of sugar added to dough depends on the process and the product, but generally varies between zero and 15% (flour basis), the former typical of a European-

style lean formula such as baguettes and the latter for sweet goods such as cinnamon rolls (Varo et al. 1979; Kulp and Ponte 2000). The most common sources of sugar are sucrose and high fructose corn syrup (Lorenz and Kulp 1995).

Table 1.3. Mono- and di-saccharide levels in flour.

component	*% flour*
glucose	0.01-0.09
fructose	<0.08
sucrose	0.1-0.33
maltose	<1.67

Compiled from Lee et al. (1959), Lee and Geddes (1959), D'Appolonia et al. (1971), Suomalainen et al. (1972), Potus et al. (1994), Martinez-Anaya (1996), and Laaksonen and Roos (2000).

Maltose content increases during the initial stages of mixing due to the action of amylolytic enzymes on damaged starch (Koch et al. 1954; Lee and Geddes 1959; Potus et al. 1994).

1.3.5 Salt

Salt (NaCl) improves the flavour of bread and slows yeast activity, thus the rate of proofing can be controlled by varying salt level (Collins 1985; Kulp and Ponte 2000). Hydrophobic interactions important to the structure of gluten are enhanced by the addition of salt (Tolstoguzov 1997), resulting in a more rigid, less sticky dough (Jenkins 1975; Collins 1985). Adding salt reduces the uptake of water by gluten (Bushuk and Mehrotra 1977a; Larsson 2002; Seguchi et al. 2003).

1.3.6 Lipids

The ‘shortening effect’ refers to an increase in loaf volume and quality from the addition of 0.7-3.0 % fat to a dough, which is standard practice (Mecham 1971; Bell et al. 1977; Pomeranz and Chung 1978; Pomeranz 1980). Lipid crystals contribute to the structural integrity of gas cell membranes and improve gas retention during mixing and the early stages of baking (Bell et al. 1977; Macritchie 1977). Fats also improve the keeping quality, softness and moistness of bread (Autio and Laurikainen 1997).

1.3.7 Improvers and Conditioners

The terms ‘improver’ and ‘conditioner’ cover a variety of refined chemicals added to dough in small quantities. For example:

- Oxidising agents convert sulphydryl groups of gluten proteins into disulphide linkages between adjacent peptides, which strengthens the dough and gives better loaf volume (Collins 1985). Examples include azodicarbonamide and potassium bromate.
- Emulsifiers and other surface-active agents improve loaf volume, crumb texture and keeping qualities (Jenkins 1975; Collins 1985). Diacetyl tartaric acid esters of monoglycerides (DATEM or DATA esters) and stearoyl-2-lactylates are common examples.
- Enzymes: α-amylase from cereal, bacterial or fungal sources retards staling in baked product and hydrolyses starch to sugars, which can take part in browning and flavour-producing reactions (Jenkins 1975; Collins 1985). Lipoxygenase, often supplied by soy flour, strengthens dough via coupled oxidation reactions with gluten and bleaches undesirable pigments (Collins 1985). Proteases are added to excessively strong flours to shorten mixing time and improve machinability (Jenkins 1975).
- Nitrogen and calcium salts ensure that yeast cells are adequately nourished during long fermentations (Collins 1985).

1.4. Overview of selected dough testing methods

1.4.1 Test baking

A wide variety of experimental methods have been brought to bear on the breadmaking process, but by far the most popular is small-scale test-baking. Loaf volumes in millilitres are often reported along with manually-scored quality parameters. As little as 2 grams of flour is required for such tests, and they are useful when sample size is limiting, such as in experimental breeding programs (Rasper and Walker 2000). Conditions must be carefully controlled to allow for the full baking potential of the flour to be exploited and to ensure intra- and inter-laboratory comparability (Bloksma 1971; Pyler 1988; Rasper and Walker 2000).

Although loaf volume is correlated with consumer acceptance, rheological properties and other breadmaking parameters (Pomeranz et al. 1970), a baking test reflects the composite effects of numerous factors operating in a complex system. As such, test baking yields little specific information about individual processes within dough, such as yeast activity and gas retention.

1.4.2 Rheological methods

Recording mixers such as the Brabender Farinograph and Mixograph are among the most widely used physical testing instruments. In most cases they continuously record the force required to rotate one or more mixing pins or heads at constant speed, and the time-force curve is referred to as a farinogram or mixogram (Bloksma 1971; Pyler 1988; Rasper and Walker 2000). The optimal water level ('water absorption') for a given flour is often defined as the amount required for a flour-water dough to reach a defined consistency, e.g. 500 Farinograph units, at the point of optimal development (Rasper and Walker 2000).

Single-point rheological testing using stress-strain instruments like the Extensigraph is also commonplace and yields parameters such as maximum resistance, resistance at a fixed extension, or extensibility, which is the distance a dough piece can be stretched before rupturing (Bloksma 1971; Pyler 1988; Rasper and Walker 2000).

Dynamic Mechanical Analysis (DMA) examines the frequency-dependent stress relaxation properties of a sample subjected to small-amplitude oscillatory stress.

Dynamic Mechanical Thermal Analysis (DMTA) operates on the same principle but also applies a temperature program to the sample.

1.4.3 Electromagnetic methods

Nuclear Magnetic Resonance (NMR) measures the absorption and emission of electromagnetic energy in a magnetic field by nuclei with nonzero spin quantum numbers, e.g. ^{1}H, ^{13}C, ^{17}O. After absorbing a radio-frequency pulse, a nucleus will relax to lower energy at a characteristic rate depending on its chemical and physical environment, so relaxation measurements can provide information on the location and surroundings of molecules containing these nuclei (Ruan and Chen 1998).

Dielectric analysis (DEA) measures the capacitance and conductance of a sample between two electrodes by analysis of the current generated when a sinusoidal voltage is applied (Laaksonen and Roos 2000). Dielectric thermal analysis (DETA) superimposes a temperature program on the measurement.

1.4.4 Thermal methods

Differential Thermal Analysis (DTA) instruments continuously measure the temperature difference between sample and reference cells during heating or cooling at a constant rate of energy input (Franks 1982). The temperature range is usually chosen to straddle a phase change in the sample, and the reference material should not undergo a phase change in that range. Suitable calibration of a DTA apparatus allows the user to estimate the amount of material undergoing phase change.

Differential scanning calorimetry (DSC) apparatus maintain reference and test samples at the same temperature during a heating run, and record the difference in the amount of heat required to do so (Franks 1982). Since the temperature scanning rate is constant, the actual quantity thus measured is the difference in specific heat between sample and reference (Franks 1982; Reid et al. 1993).

1.4.5 Manometric methods

A widely-used measure of yeast activity in dough is the volume or pressure of gas generated by a dough proofing in a closed container (‘gassing power’). Pressure gauges and liquid manometers have largely been replaced by automated apparatus such as the Risograph and Fermentograph, which record gas production from up to 12 proofing doughs simultaneously.

The total pressure or volume of gas generated during 2 to 3 hours of proofing is often reported, and cumulative volume vs. time plots ('gassing curves') are sometimes published. Meric et al. (1995) found that mean volume (area under the gassing curve divided by time) was a better measure of gas production kinetics than total gas volume after a given time or time to proof to a given volume. They noted that mean volume favourably weighted rapid initial gas production. Cumulative volume data can be converted to gas production rates, but these are seldom found in literature.

The gassing power of fresh doughs is not directly comparable with that of colder frozen-thawed doughs because the temperature difference alone can account for ~15% difference in gassing power (Kline and Sugihara 1968; Meric et al. 1995).

1.4.6 Extraction of yeast metabolites

Sugars can be extracted from dough with water or organic solvents, at room temperature or elevated temperature. The American Association of Cereal Chemists (AACC) approved method 80-04 "Simple sugars in cereal products by HPLC" (American Association of Cereal Chemists 1995) involves shaking a 10g sample in 100ml water for 20 minutes.

Several workers have extracted sugars from dough in hot ethanolic solutions (Koch et al. 1954; Tang et al. 1972; Varo et al. 1979; Langemeier and Rogers 1995). Others have used water (Loenner and Preve-Akesson 1988; Lefebvre et al. 2002) or acetone and methanol:water (1:1) (Rouzaud and Martinez-Anaya 1993).

No methods for analysis of ethanol in dough have been approved by the AACC. Some workers have extracted dough in water, distilled the aqueous extract and measured ethanol in the distillate by gravimetric methods (Blish and Hughes 1932) or other volatile organic compounds by gas chromatography (Frasse et al. 1993). Others have measured ethanol in aqueous dough extract with enzymatic test kits (Saalfeld and Freund 1998; Lefebvre et al. 2002). Tomas and Harren (2001) developed a method in which nitrogen gas was passed over the surface of a thin dough piece, and the entrained ethanol and acetaldehyde were measured with a photoacoustic spectrometer.

It is sometimes necessary to remove protein from dough extracts, particularly when chromatographic assays are used. Protein can be precipitated by heating (Varo et al. 1979; Potus et al. 1994; Langemeier and Rogers 1995) but extracts are more often treated with chemical denaturants such as $HClO_4$ or Carrez reagents (potassium II

hexaferrocyanate and zinc sulphate) (Saalfeld and Freund 1998; Lefebvre et al. 2002). Carrez reagents give more efficient protein removal than $HClO_4$ and avoid hydrolysis of disaccharides (Lefebvre et al. 2002).

1.4.7 Enzymatic assay of yeast metabolites

Sugars and ethanol in dough extracts have been measured by HPLC (Varo et al. 1979; Potus et al. 1994; Langemeier and Rogers 1995; Lefebvre et al. 2002) and with enzymatic assays (Saalfeld and Freund 1998; Lefebvre et al. 2002). Lefebvre et al. (2002) found no significant difference between results obtained with the two methods.

Principles of enzymatic assays are described in Bergmeyer et al. (1984) and these descriptions are reproduced in part below.

D-*Glucose*

D-glucose is phosphorylated to D-glucose-6-phosphate (G6P) by the enzyme hexokinase (HK) (equation 1.1). The conversion is catalysed by hydrolysis of ATP.

$$D\text{-}Glucose + ATP \xrightarrow{HK} G6P + ADP \qquad 1.1$$

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G6P is oxidized by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH).

$$G6P + NADP^+ \xrightarrow{G6P-DH} D-gluconate-6-phosphate + NADPH + H^+ \qquad 1.2$$

The amount of NADPH formed is equimolar to the amount of D-glucose in the sample. Formation of NADPH is measured by the increase in light absorbance at 340nm, and glucose concentration is deduced NADPH concentration.

D-Fructose

Hexokinase catalyses the phosphorylation of both glucose (equation 1.1) and fructose (equation 1.3).

$$D\text{ - }Fructose + ATP \xrightarrow{HK} F6P + ADP \qquad 1.3$$

In the fructose assay, fructose-6-phosphate (F6P) undergoes isomerisation to glucose-6-phosphate catalysed by phosphoglucose isomerase (PGI):

$$F6P \xrightarrow{PGI} G6P \qquad 1.4$$

The G6P from both glucose phosphorylation and F6P isomerisation is quantified by oxidizing to D-gluconate-6-phosphate (equation 1.2). The fructose assay measures both glucose and fructose, so glucose must measured in a separate assay and subtracted to determine fructose concentration.

Sucrose

Sucrose is hydrolysed to D-glucose and D-fructose by the enzyme beta-fructosidase (invertase) at pH 4.6:

$$Sucrose + H_2O \xrightarrow{\beta-fructosidase} D-glucose + D-fructose \qquad 1.5$$

The amount of D-glucose released is equimolar to the amount of sucrose. Total D-glucose is quantified as above and sucrose concentration deduced after subtraction of glucose from sources other than sucrose hydrolysis.

Maltose

Maltose is hydrolysed to glucose by the enzyme enzyme α-glucosidase (maltase) at pH 6.6:

$$Maltose + H_2O \xrightarrow{\alpha-glucosidase} 2D-glucose \qquad 1.6$$

Alpha-glucosidase also hydrolyses sucrose, and the maltose concentration is deduced from total glucose by subtracting sucrose and glucose assay results and dividing by two.

1.5. Factors affecting the shelf life of frozen dough

Frozen doughs must be thawed and proofed before being baked, and proofing must be quick enough to meet the needs of the consumer. The shelf life of frozen dough is limited by the decline of proofing power with extended frozen storage. This has been attributed to both diminished gas-holding properties and a fall in gas production, i.e. loss of yeast activity (Casey and Foy 1995).

The shelf life of frozen dough is usually gauged by the time taken for a dough to proof to a given height (proof time), the height attained by a dough after a given proofing time (proof height) or the volume of a loaf produced in small-scale test baking (loaf volume). These parameters are influenced by both gas retention and gas production. Yeast activity can be measured independently of gas retention as the pressure or volume of gas generated by dough proofing in a closed container.

1.5.1 Loss of gas retention

Frozen storage and freeze-thaw cycles lead to significant changes in the rheological properties of dough ('slackening'), which negatively impact on the dough's ability to retain gas produced by yeast. Some studies have found an increase in maximum resistance relative to extensibility with frozen storage or freeze-thaw cycles (Varriano-Marston et al. 1980; Wolt and D'Appolonia 1984a; Bhattacharya et al. 2003), whereas others reported the opposite tendency (Inoue and Bushuk 1991, 1992; Inoue et al. 1994). Oxidants are often included in frozen dough formulations to strengthen the dough and compensate for slackening (Casey and Foy 1995; Inoue and Bushuk 1996).

Work on the impact of yeast on dough rheology has been equivocal: some researchers report a link between pre-freezing or post-thawing yeast activity and slackening during frozen storage (Inoue and Bushuk 1991; Inoue et al. 1994) while others report similar rheological changes in yeasted and unyeasted doughs (Varriano-Marston et al. 1980). Kline and Sugihara (1968) suggested that rheological changes in yeasted frozen doughs resulted from disruption of gluten disulphide bonds by reducing substances leached from dead yeast cells. However this was discounted by other researchers, who found no change in the free sulphydryl content of dough during frozen storage (Wolt and D'Appolonia 1984a). Incorporating dead yeast cells (Autio and Sinda 1992) or leachate from frozen yeast (Wolt and D'Appolonia 1984a) into frozen unyeasted doughs did not reproduce the rheological effects of frozen storage on yeasted doughs.

Loss of gas retention has been attributed to physical disruption of the dough matrix caused by ice recrystallisation (Varriano-Marston et al. 1980; Berglund et al. 1990; Berglund et al. 1991; El-Hady et al. 1996; Zounis et al. 2002a; Zounis et al. 2002b). The progressive dehydration of gluten as water migrates into ice crystals has also been proposed (Varriano-Marston et al. 1980; Berglund et al. 1991; Räsänen et al. 1997; Räsänen et al. 1998; Lu and Grant 1999; Bhattacharya et al. 2003). Some authors have reported that reduced-water doughs were more stable in frozen storage (El-Hady et al. 1996; Räsänen 1998), but others have found no improvement (Gelinas et al. 1995).

The incorporation of freezing point depressants such as sucrose, ethanol and polyhydroxy alcohols to inhibit ice formation and soften the dough for rapid thawing has been the subject of several patents (Lindstrom and Slade 1983; Lonergan et al. 1997, 1998; Goedeken et al. 2003). Hydrocolloids such as maltodextrin and xanthan gum also reduce the deleterious effects of freezing and frozen storage (Felske and Silva 1988; Räsänen 1998; Lonergan et al. 1998), though their influence on ice crystal formation and growth is poorly understood (Blond 1985, 1988; Budiaman and Fennema 1987a, b).

Packaging frozen dough in a CO_2-enriched atmosphere decreases proofing time of the thawed dough (Lonergan et al. 1997, 1988). Frozen storage in air results in the loss of dissolved carbon dioxide from dough as an equilibrium with the surrounding atmosphere (mainly nitrogen) is established. When the loss of CO_2 is prevented by packaging in a CO_2-enriched environment, more remains dissolved in the dough, and there is a greater driving force for CO_2 produced during proofing to evaporate from solution and expand the dough (Lonergan and McIntyre 2003).

1.5.2 Loss of yeast activity

Although slackening contributes to the decline in proofing power, it is generally accepted that loss of yeast activity is the biggest problem in frozen manufacture (Kline and Sugihara 1968; El-Hady et al. 1996). Freezing and thawing without frozen storage does not reduce gassing power substantially, although apparent gassing power differences between fresh and thawed doughs may arise from temperature differences (Tanaka and Miyatake 1975; Meric et al. 1995). Doughs frozen immediately after mixing retain >75% of their gassing power after frozen storage for up to 15 weeks (Kline and Sugihara 1968). If doughs are pre-fermented before freezing the loss in gassing power is much faster and shelf life is shortened (Godkin and Cathcart 1949; Kline and Sugihara 1968; Hsu et al. 1979a).

Some authors have reported that pre-fermentation for 30-60 minutes has a moderately detrimental effect on post-thaw gassing power, which drops off faster with longer fermentations (Kline and Sugihara 1968; Meric et al. 1995; Nemeth et al. 1996; Almeida and Pais 1996; Kenny et al. 2001). For example, Meric et al. (1995) found that doughs fermented 30 or 60 minutes retained 88-100% of gassing power after 3-4 weeks' frozen storage, compared with 62-73% retention for doughs fermented 80 or 120 minutes. The effect of fermentation varies widely among yeast strains (Hino et al. 1987; Hino et al. 1990; Baguena et al. 1991; Almeida and Pais 1996).

Although there is a general acceptance that pre-fermentation should be minimised in frozen dough manufacture, the mechanism by which pre-fermentation accelerates the loss of gassing power is not well understood.

The effect of pre-fermentation can be partly mitigated by adding yeast in the later stages of mixing (Dubois and Blockolsky 1986; Neyreneuf and Van der Plaat 1991) or mixing at reduced temperature (Neyreneuf and Van der Plaat 1991; Meric et al. 1995; Kenny et al. 2001; Zounis et al. 2002b). However colder doughs require more energy and longer mixing time to adequately develop gluten (Neyreneuf and Van der Plaat 1991; Zounis et al. 2002b).

Compressed yeast generally outperforms dried yeast in frozen dough (Kline and Sugihara 1968; Wolt and D'Appolonia 1984b; Neyreneuf and Van der Plaat 1991; El-Hady et al. 1996) and shows equivalent performance to liquid yeast (Gelinas et al. 1994). Dried yeasts take longer to become active, which reduces the extent of pre-fermentation (El-Hady et al. 1996), but the drying process renders yeast more

vulnerable to freezing damage (Wolt and D'Appolonia 1984b; Rasper and Walker 2000).

It is difficult to generalise about the impact of the freezing process on frozen dough shelf life because of the range of dough geometries and experimental conditions reported. Some authors report small and erratic effects of freezing protocol (Lehmann and Dreese 1981). Others have found that blast freezing in air at –20˚C gives better retention of gassing power than with lower temperatures, but findings are contradictory with regard to the effect of air velocity (El-Hady et al. 1996; Le Bail et al. 1997; Le Bail et al. 1998; Le Bail et al. 1999; Havet et al. 2000). The local freezing rate varies throughout a dough piece, which can give rise to differences in yeast activity in different parts of the dough (Le Bail et al. 1997; Havet et al. 2000).

Freezing to –40˚C and below or transferring doughs to storage at a temperature below the freezing temperature are detrimental to frozen dough performance (Hsu et al. 1979b). Fluctuations in storage temperature accelerate the loss of yeast activity (Hsu et al. 1979b; Le Bail et al. 1999). Doughs frozen at -40˚C to -120˚C using a mixture of air and boiling liquid nitrogen retain less gassing power the lower the freezing temperature (Neyreneuf and Delpuech 1993; Masovic and Jankovic 2002).

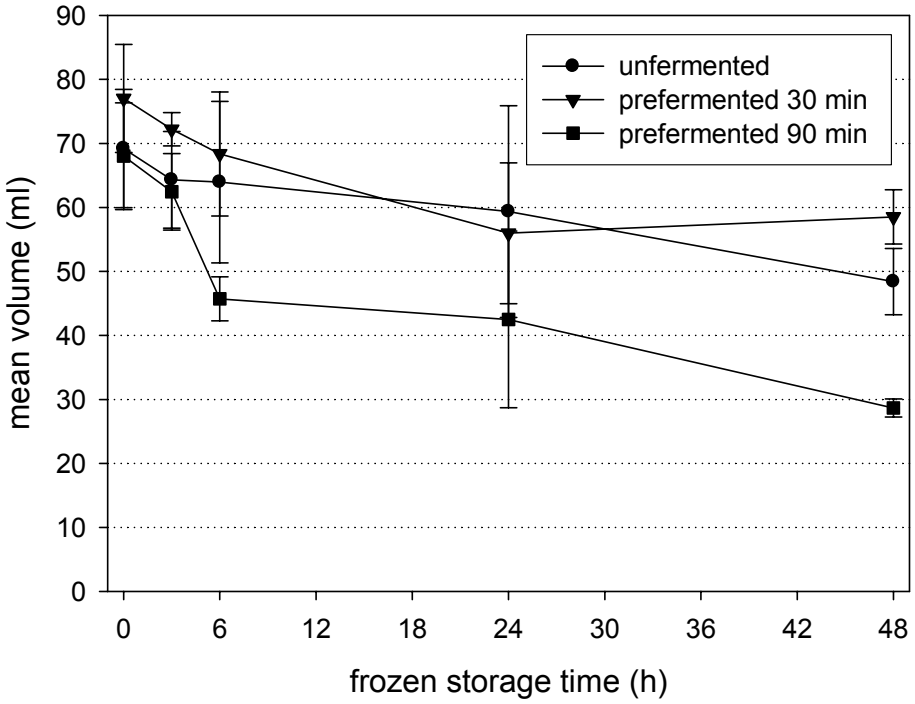
Long thawing times give shorter final proofing times (Dubois and Blockolsky 1986; Reid 1998), probably because yeast cells are active at thawing temperatures and some degree of pre-proofing occurs.

1.5.3 Frozen dough studies in New Zealand

Preliminary work in this laboratory (Shannon 2003) established a relationship between prefermentation time and gassing power of frozen doughs. Data from that work were used to calculate mean volumes (Appendix A3) according to the method of Meric et al. (1995).

Doughs pre-fermented for 90 minutes had significantly lower gassing power after 6 and 48 h frozen storage than dough pre-fermented for a shorter time (Table 1.4 and Figure 1.3). The gassing power of 30 minute pre-fermented doughs was significantly higher than other doughs after 3 and 48 h frozen storage.

Figure 1.3. Effect of pre-fermentation on gassing power of frozen dough.



Doughs were pre-fermented in bulk (1kg) at room temperature prior to dividing and packaging. Packaged doughs were frozen at -15˚C for 1 h and stored at -20˚C for the required time then thawed at 0˚C for 1 h. Gassing power of 60 minute pre-fermented doughs not shown for clarity. Vertical bars are standard deviations of 1 or 2 replicate determinations on 2 or 3 independent doughs. Data from Shannon (2003).

Table 1.4. Gassing power of pre-fermented frozen doughs.

fermentation time (min)	Time in frozen storage (-20˚C)																							
	fresh				1 h				3h				6h				24h				48h			
	V_M[A]	SD[B]	n[C]		V_m	SD	n		V_m	SD	n		V_m	SD	n		V_m	SD	n		V_m	SD	n	
0	76.13	11.77	6	a[D]	69.19	9.23	4	a	64.30	7.54	6	b	63.96	12.62	6	a	59.35	16.54	5	a	48.41	5.18	4	b
30	89.85	5.71	6	a	77.04	8.43	5	a	72.22	2.62	6	a	68.37	9.69	6	a	55.97	11.01	4	a	55.52	4.24	4	a
60	83.33	13.67	6	a	76.27	7.61	6	a	70.17	4.06	6	ab	62.83	1.81	6	a	47.74	4.71	4	a	41.35	6.46	4	b
90	86.03	4.16	6	a	68.00	8.36	6	a	62.44	5.99	6	b	45.72	3.43	4	b	42.49	13.81	3	a	28.67	1.43	3	c

Doughs (1kg) were pre-fermented in bulk at room temperature (approximately 20˚C) for 0, 30, 60 or 90 minutes and frozen at -15˚C for 1 h. After storage at -20˚C doughs were thawed at 0˚C for 1 h and gassing power measured in a Risograph at 30˚C.

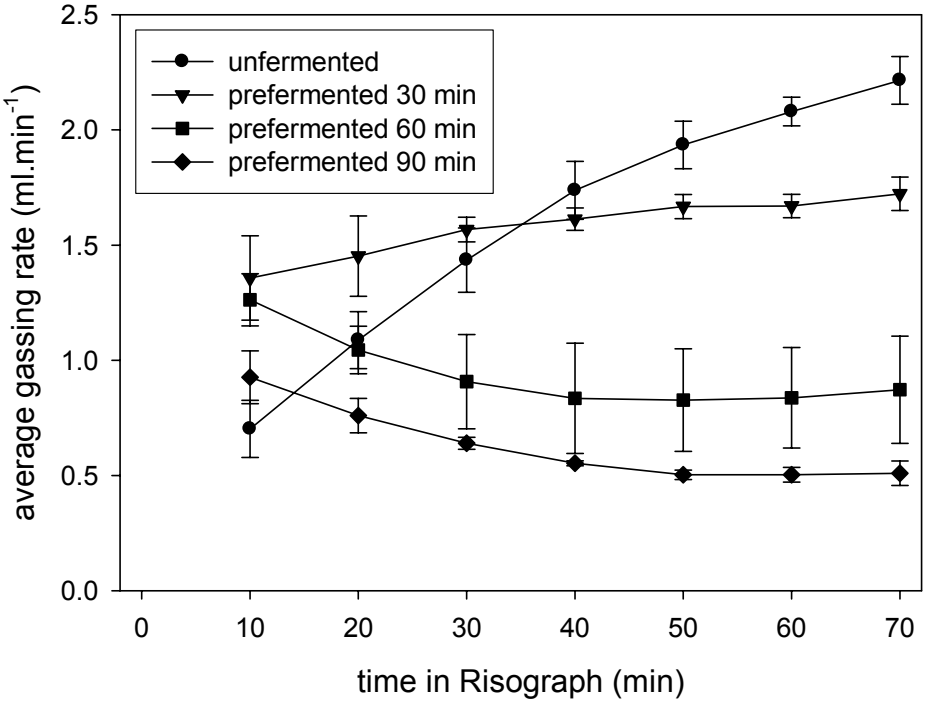
Gassing power of frozen and thawed dough was not directly comparable with fresh doughs due to temperature differences – unfrozen doughs were at room temperature when placed in the Risograph whereas thawed doughs were at 0˚C.

Data from Shannon (2003).

A. mean volume
B. standard deviation
C. number of replicates
D. Lower case letters indicate significant differences (p=0.05) down columns.

The gassing rates of doughs stored 48 h (Figure 1.4) illustrate why 30 minute pre-fermented doughs outperformed all other doughs and 90 minute pre-fermented doughs had inferior gas production.

Figure 1.4. Effect of pre-fermentation on gassing rates of doughs stored 48 h at -20˚C



Doughs were prefermented in bulk (1kg) at room temperature. The first ten minutes of data were discarded to allow for thermal expansion of air in the Risograph can. Data points are the mean of 1 or 2 replicate determinations on 2 or 3 independent doughs. Vertical bars are standard deviations. Data from Shannon (2003).

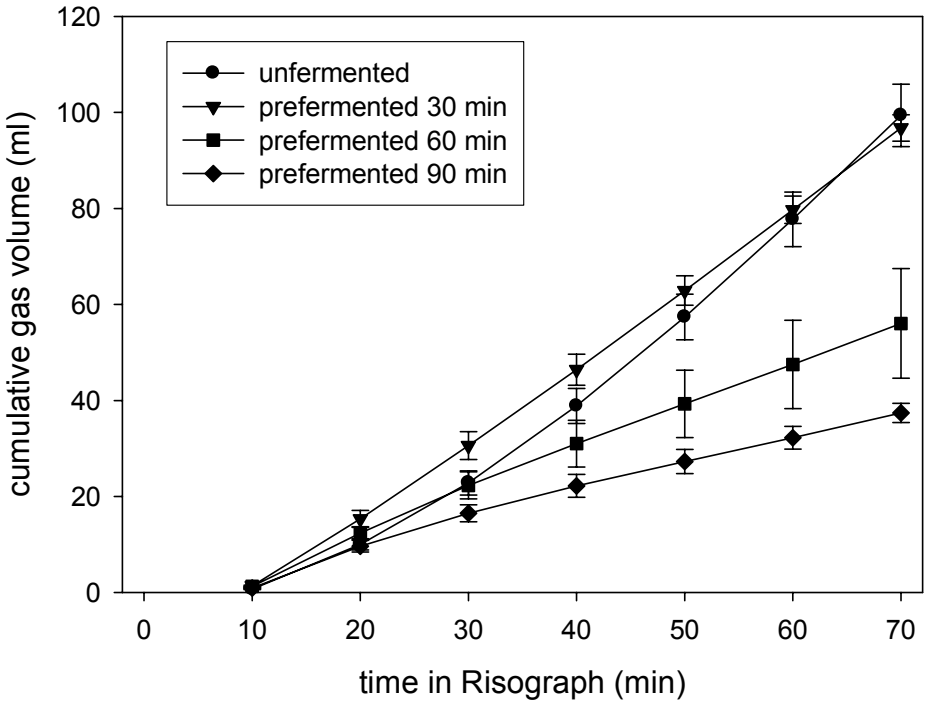
Unfermented doughs proofed more slowly than pre-fermented doughs at first, but gassing rate accelerated rapidly and overtook them within 30 minutes. After 50-60 minutes unfermented doughs and 30 minute pre-fermented doughs had produced equal volumes of gas (Figure 1.5), but mean volume was higher in the pre-fermented doughs (Table 1.4) because of rapid initial gas production.

During pre-fermentation carbon dioxide excreted by yeast cells dissolved in the dough, and evaporation of dissolved CO_2 into gas cells was slow while the dough was far from saturated (Hibberd and Parker 1976). Pre-fermented doughs contained more

dissolved CO_2, and more of the CO_2 excreted by yeast cells during post-thawing fermentation evaporated into gas cells, expanding the dough. This would explain the higher initial gas production in pre-fermented doughs.

However a weak increase in gas production by the 30 minute pre-fermented doughs and decline in gassing rate with longer pre-fermentation suggests that yeast cells in pre-fermented doughs were more vulnerable to freezing damage.

Figure 1.5. Effect of pre-fermentation on cumulative gas production of doughs stored 48h at -18˚C.



Doughs were prefermented in bulk (1kg) at room temperature. The first ten minutes of data were discarded to allow for thermal expansion of air in the Risograph can. Data points are the mean of 1 or 2 replicate determinations on 2 or 3 independent doughs. Vertical bars are standard deviations.

The gassing power of frozen doughs declined over a matter of days rather than weeks, as has been observed in other studies (Kline and Sugihara 1968; Meric et al. 1995). This may be attributable to differences in dough formulation and manufacture, especially yeast strain (Hino et al. 1990). The New Zealand yeast used here, although recommended by the manufacturer for frozen dough applications (personal communication from Jeremy Paterson, New Zealand Food Industries Ltd., 2003) appeared to perform differently from yeasts manufactured in other countries.

Gas production was measured during the first hour of post-thaw proofing, whereas others have proofed doughs for up to three hours in gassing power measurements (Kline and Sugihara 1968; Meric et al. 1995). The focus on initial gassing rate here was emphasized by the mean volume calculation, which favourably weighted rapid initial gas production (Meric et al. 1995).

1.6. Sugar metabolism of baker's yeast

1.6.1 Sugars taken up by yeast

Relatively few sugars are good substrates for *Saccharomyces cerevisiae* fermentation, namely the monosaccharides glucose, fructose, mannose and galactose and disaccharides sucrose (α-D-Glc-*p*-(1→2)-β-D-Fru-*f*), maltose (α-D-Glc-*p*-(1→4)- D-Glc-*p*) and α,α-trehalose (α-D-Glc-*p*-(1→1)-α-D-Glc-*p*) (Chen and Chiger 1985; Walker 1998; Rasper and Walker 2000). Constitutive uptake of maltotriose (α-D-Glc-*p*-(1→4)-α-D-Glc-*p*-(1→4)-D-Glc-*p*) has been reported, and the trisaccharide raffinose (α-D-Gal-*p*-(1→6)-α-D-Glc-*p*-(1→2)-β-D-Fru-*f*) is partially fermented, leaving melibiose (α-D-Gal-*p*-(1→6)-D-Glc-*p*) as a residue (Chen and Chiger 1985; Walker 1998).

The main yeast substrates in dough are glucose, fructose, sucrose and maltose. Invertase enzymes on the surface of yeast cells rapidly hydrolyse sucrose to glucose and fructose during mixing and the early stages of fermentation (Koch et al. 1954; Varo et al. 1979; Rogers and Langemeier 1995). Yeast enzymes also hydrolyse oligosaccharides of glucose and fructose (glucofructans) originating from flour (Koch et al. 1954; Lee and Geddes 1959; Suomalainen et al. 1972; Potus et al. 1994).

Production of baker's yeast has been well-reviewed in the literature (Harrison 1970; Chen and Chiger 1985; Evans 1990; Reed and Nagodawithana 1991). The most widely-used carbohydrate source is beet or cane molasses, which is rich in sucrose, glucose and fructose and contains some raffinose (Evans 1990; Reed and Nagodawithana 1991).

1.6.2 Sugar uptake kinetics

Sugars are quite polar due to their hydroxyl groups and do not partition easily into the yeast cell membrane, which is a lipid bilayer. Trans-membrane sugar carriers have not been isolated to date, and debate continues regarding the regulation, structure and functional properties of single or multiple sugar transport systems.

Sugar uptake by *Saccharomyces cerevisiae* can be considered as a series of equilibria involving sugar molecules and sugar carriers spanning the cell membrane:

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SC \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C + S' \qquad 1.7$$

Where *S* is the sugar outside the cell, *C* is the free carrier, *SC* is sugar molecule-carrier complex and *S'* is sugar inside the cell. The concentration of *SC* is assumed to be constant, the so-called steady-state assumption, and solving for the rate at which *S'* is released (Cornish-Bowden 1995) gives an equation of the following form:

$$\frac{dS'}{dt} = \frac{k_1 k_2 [C]_0 [S] - k_{-1} k_{-2} [C]_0 [S']}{k_{-1} + k_2 + k_1 [S] + k_{-2} [S']} \qquad 1.8$$

Where $[C]_0$ is the initial concentration of free carrier. Equation 1.8 can be simplified under two circumstances:

- *[S']* is small, which is true when uptake first begins or if sugar inside the cell is eliminated or modified by metabolic reactions such as phosphorylation (Bisson and Fraenkel 1983)
- k_{-2} is small, i.e. low affinity between sugar and carrier on the inside surface of the membrane (Wilbrandt and Rosenberg 1961)

Simplification gives a hyperbolic equation analogous to that used by Michaelis and Menten (1913) to model sucrose inversion and by Monod (1949) to fit bacterial growth curves:

$$V = \frac{X.V_{max}.S}{S + K_m} \qquad 1.9$$

V	rate of sugar uptake	[mmol.L^{-1}.h^{-1}]
V_{max}	maximum specific rate of sugar uptake	[mmol.(g biomass)$^{-1}$.h^{-1}]
X	biomass concentration	[g.L^{-1}]
S	sugar concentration	[mmol.L^{-1}]
K_m	affinity constant	[mmol.L^{-1}]

A more complicated three-step mechanism in which the carrier-sugar complex moves from one side of the membrane to the other (Orlowski 1987) adds an extra equilibrium step to 1.7. However the rate equation is of the same form as 1.8 (Cornish-Bowden 1995).

This approach to predicting sugar consumption requires the implicit assumption that uptake rather than glycolysis is the limiting step in sugar utilisation. Evidence for this comes from findings that glycolytic enzyme activities are relatively constant at different uptake rates (Weusthuis et al. 1993) and the intracellular concentration of free glucose is low (Liao et al. 1988; Gancedo and Serrano 1989).

V_{max} and K_m are usually determined from plots of *1/V* vs. *1/S* known as Lineweaver-Burk plots (Peinado and Loureiro-Dias 1986; D'Amore et al. 1989; Medintz et al. 1996; Mwesigye and Barford 1996), *S/V* vs. *S* or Eadie-Hofstee plots (Lovgren and Hautera 1977; Busturia and Lagunas 1985, 1986; Salmon et al. 1993), other linear plots (Cornish-Bowden 1995) or with computer-assisted nonlinear regression (Wrede et al. 1992; Fuhrmann and Volker 1993; Reinhardt et al. 1997). Lineweaver-Burk or 'double-reciprocal' plots have long been acknowledged as unreliable due to their weighting of lower substrate concentrations (Dowd and Riggs 1965; Bisson and Fraenkel 1983; Cornish-Bowden 1995; Marangoni 2003) but are widely encountered nonetheless.

1.6.2.1. Monosaccharides

Monosaccharides cross the yeast cell membrane by facilitated diffusion (Siro and Loevgren 1979; Lagunas 1993; Walker 1998). There is evidence for two or more hexose uptake systems with substrate affinities an order of magnitude apart (Serrano and De la Fuente 1974; Bisson and Fraenkel 1983; Busturia and Lagunas 1986). At low concentration the affinity for sugar is high (i.e. low K_m) but when substrate is more readily available affinity is low (i.e. high K_m).

The high-affinity system may be linked with the phosphorylation of sugars (Serrano and De la Fuente 1974; Bisson and Fraenkel 1983), but conflicting results have been reported in this regard (Lagunas 1993). Several authors have postulated that the two kinetic regimes result from a single carrier interconverting between two states (Serrano and De la Fuente 1974; Barford et al. 1992b). Others argue that low-affinity uptake is due to passive diffusion (Fuhrmann and Volker 1992; Fuhrmann and Volker 1993), but this has been disputed (Gamo et al. 1995; Coons et al. 1995).

Glucose and fructose uptake systems have much in common: uptake rates of either sugar alone are very similar (Serrano and De la Fuente 1974; D'Amore et al. 1989), uptake of one sugar is affected by the presence of the other (Waley 1981; D'Amore et al. 1989), there is no lag between uptake of glucose followed by fructose (Orlowski and Barford 1987), and uptake kinetics for one sugar are unaffected by adaptation on the other (D'Amore et al. 1989).

However a completely common system is unlikely on account of preferential uptake of glucose (Orlowski and Barford 1987; D'Amore et al. 1989) and higher kinetic affinity (i.e. lower K_m) for glucose than fructose (Serrano and De la Fuente 1974; Orlowski and Barford 1987; D'Amore et al. 1989).

1.6.2.2. Disaccharides

Disaccharides are either hydrolysed extracellularly or actively transported across the cell membrane (Siro and Loevgren 1979; Peinado and Loureiro-Dias 1986; Lagunas 1993; Walker 1998).

Most workers report that maltose enters the cell via permeases on the cell membrane surface and is hydrolysed by intracellular maltase (α-glucosidase) (Lovgren and Hautera 1977; Siro and Loevgren 1979; Peinado and Loureiro-Dias 1986; Lagunas 1993; Walker 1998). In some cases two-component maltose transport systems have been reported (Busturia and Lagunas 1985), but the low-affinity component may be an experimental artifact due to nonspecific binding of maltose at the plasma membrane or cell wall (Benito and Lagunas 1992).

Invertase enzymes (beta-fructosidase) on the outside surface of yeast cells are thought to rapidly hydrolyse sucrose to glucose and fructose independent of sugar uptake or metabolism (Roman 1957; Chen and Chiger 1985; Lagunas 1993). In support of this, yeasted doughs and brewing worts containing added sucrose showed a rapid disappearance of sucrose, concomitant with increases in glucose and fructose (Koch et al. 1954; Varo et al. 1979; D'Amore et al. 1989; Rogers and Langemeier 1995). Sucrose is not hydrolysed in unyeasted doughs (Potus et al. 1994). Some workers have reported that sucrose can also be transported directly into the cell (Orlowski 1987; Barford et al. 1992b; Lagunas 1993), possibly via maltose carriers (Mwesigye and Barford 1996; Stambuk et al. 2000; Batista et al. 2004).

Growth on molasses during baker's yeast production causes cells to become adapted to consuming sucrose and its hydrolysis products. Sucrose-adapted cultures often show a short lag between depletion of sucrose, glucose and fructose and uptake of maltose (Koch et al. 1954; Tang et al. 1972; Suomalainen et al. 1972; Mwesigye and Barford 1996). In extended fermentations, exhaustion of monosaccharides is accompanied by a dip in gas production while cells adapt to maltose utilisation (Koch et al. 1954). Invertase levels are similar in maltose- and sucrose-grown cells, but maltase is much lower in sucrose-grown cells (Mwesigye and Barford 1996), so the lag may be required for synthesis of the enzymes necessary to metabolise maltose.

The repression of maltose metabolism in yeast by sucrose, glucose and fructose is well-known and has been widely investigated and reviewed (Lovgren and Hautera 1977; Siro and Loevgren 1979; Busturia and Lagunas 1985; Peinado and Loureiro-Dias 1986; Ernandes et al. 1992; Medintz et al. 1996; Crumplen et al. 1996; Verstrepen et al. 2004). Repression occurs via regulation of transcription and/or translation of genes coding for (a) maltose permeases, (b) maltase and (c) an activator of transcription (Lovgren and Hautera 1977; Siro and Loevgren 1979; Lagunas 1993). Some studies have found that glucose repression requires protein synthesis (Busturia and Lagunas 1985) but others have observed glucose repression when protein synthesis is inhibited (Peinado and Loureiro-Dias 1986).

Inhibition of maltose uptake by glucose and fructose follows classical noncompetitive inhibition kinetics (decrease in V_{max} but not K_m) (Lovgren and Hautera 1977). Culturing yeast in maltose-rich medium can counteract glucose repression (Ernandes et al. 1993).

There is poor correspondence between maltase activity and maltose uptake or CO_2 production in broth cultures (Lovgren and Hautera 1977) but correlations across different batches of baker's yeast have been reported (Hautera and Loevgren 1975). Maltase activity shows no relation to leavening ability in dough, which may be due to repressive concentrations of glucose (Suomalainen et al. 1972; Hautera and Loevgren 1975) or a bottleneck at maltose permease.

1.6.3 Metabolic reactions in sugar catabolism

Saccharomyces cerevisiae can catabolise glucose and fructose via oxidative (respiratory) and/or reductive (fermentative) pathways according to the overall reactions shown in equations 1.10 and 1.11.

Oxidative sugar catabolism:

$$C_6H_{12}O_6 + 6\ O_2 + 30ADP + 30P_i \longrightarrow 6CO_2 + 6H_2O + 30ATP \qquad 1.10$$

Reductive sugar catabolism:

$$C_6H_{12}O_6 + 2ADP + 2P_i \longrightarrow 2CO_2 + 2C_2H_5OH + 2ATP \qquad 1.11$$

P_i = inorganic phosphate, ATP = adenosine triphosphate, ADP = adenosine diphosphate. $C_6H_{12}O_6$ is glucose or fructose.

Glucose and fructose both enter the glycolytic pathway, which produces two molecules of pyruvate per molecule of hexose (Gancedo and Serrano 1989). When oxygen is available pyruvate is oxidised in the TCA cycle, but in anaerobic conditions it is decarboxylated to acetaldehyde and reduced to ethanol in order to regenerate NAD^+ (Gancedo and Serrano 1989).

When sugar concentration is very low (<5 $mmol.L^{-1}$ glucose (Lagunas, 1979)) and oxygen is non-limiting, oxidative metabolism predominates (Walker 1998). Baker's yeast is produced under conditions of high dissolved oxygen content and restricted sugar supply, which favour oxidative metabolism and give high biomass yields (Harrison 1970; Chen and Chiger 1985). At higher sugar concentration reductive pathways predominate irrespective of oxygen concentration. This phenomenon has been attributed to either saturation of oxidative capacity (Barford and Hall 1981; Sonnleitner and Kapelli 1986; Barford et al. 1992b) or repression of oxidative metabolism by sugars, i.e. the 'Crabtree effect' (Verstrepen et al. 2004; Thierie 2004).

It has been stated that dough is an essentially anaerobic environment (Liao et al. 1988; Van Hoek et al. 2000), but the oxygen status of dough has not been experimentally determined.

Although the biomass yield from reductive metabolism is low, some workers have found an increase in yeast cell numbers in dough during the course of fermentation,

e.g. 37% increase over 3.5 hours (Thorn and Ross 1960), 29-88% increase over 6 hours (Hoffman et al. 1941), 70% increase over 2 hours (Akdogan and Ozilgen 1992). Separation of yeast cells from the dough matrix is problematic (Godkin and Cathcart 1949) and counts of viable (i.e. colony-forming) or living cells often do not correspond with gassing power (Godkin and Cathcart 1949; Kline and Sugihara 1968; Autio and Mattila-Sandholm 1992; Almeida and Pais 1996).

Optimal fermentation temperature for commercial yeasts is around 38˚C, but the effect of temperature on yeast activity depends on the type of dough (Mahoney and Foy 2003). Literature reports cover the effect of temperature on gas production in dough (Chiotellis and Campbell 2003b), cell division in dough (Akdogan and Ozilgen 1992), gas production of liquid ferments (Pyler 1988), ethanol accumulation in dough (Saalfeld and Freund 1998) and the activity of commercial baker's yeast (Heckelmann 1993).

Carbon dioxide excreted by yeast cells in fermenting dough initially dissolves and reacts in aqueous solution to form carbonic acid according to equations 1.12 to 1.14.

$$CO_2\ (g) + H_2O\ (l) \rightleftharpoons H_2CO_3\ (aq) \qquad 1.12$$

$$H_2CO_3 + H_2O \rightleftharpoons HCO_3^- + H_3O^+ \qquad K_a = 4.57 \times 10^{-7} \qquad 1.13$$

$$HCO_3^- + H_2O \rightleftharpoons CO_3^{2-} + H_3O^+ \qquad K_a = 5.59 \times 10^{-11} \qquad 1.14$$

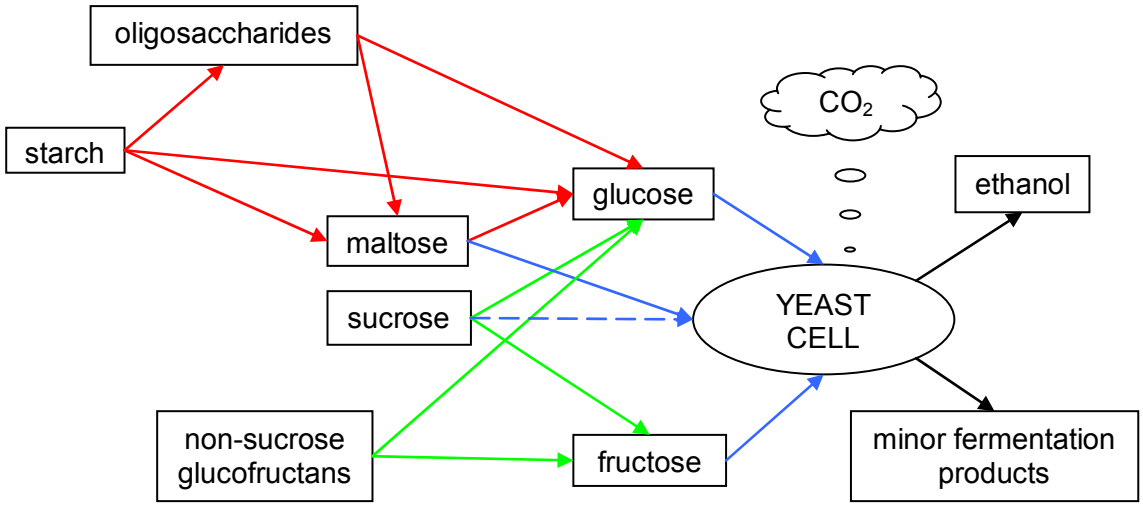
Carbon dioxide production and the excretion of organic acids in fermenting dough leads to a decline in pH from about 6 to 4.8-5.5 at the end of fermentation (Blish and Hughes 1932; Chen and Chiger 1985; Reed and Nagodawithana 1991; Baguena et al. 1991), or somewhat lower for sourdoughs (Bruinsma and Giesenschlag 1984). Baker's yeast activity is relatively constant over the pH range encountered in dough (Cooper and Reed 1968; Harrison 1970; Reed and Nagodawithana 1991). Mathematical modelling studies suggest that dissolved CO_2 begins to evaporate into air bubbles in the dough before saturation is reached (Chiotellis and Campbell 2003a, 2003b).

1.6.4 Sugar sources and sinks in dough

Yeast substrates in dough arise from several different sources, as shown in Figure 1.6. Red lines represent the actions of amylolytic flour and microbial enzymes, which liberate maltose, glucose and oligosaccharides (section 1.3).

Blue lines show the uptake of mono- and disaccharides by yeast cells. There is some evidence for uptake of intact sucrose molecules (dashed line) and extracellular hydrolysis of maltose, but most authors have concluded that sucrose is hydrolysed by pericellular invertases and maltose is transported across the cell membrane and hydrolysed intracellularly (section 1.6).

Figure 1.6. Yeast metabolite sources in dough. Line colours are explained in the text.



Green lines indicate the hydrolytic action of yeast enzymes on sucrose from endogenous or exogenous sources and non-sucrose glucofructans in flour. The identity of non-sucrose glucofructans is not well documented, but raffinose (α-Gal-*p*-(1→6)-α-Glc-*p*-(1→2)-β-Fru-*f*), gluco-difructose (1-kestose) and maltotriose have been found in flour and dough (Koch et al. 1954; Suomalainen et al. 1972; Pomeranz 1988).

Yeasted doughs contain more glucose and fructose immediately after mixing than unyeasted doughs, which is due to the action of yeast enzymes on glucofructans, especially sucrose (Potus et al. 1994). *Saccharomyces cerevisiae* beta-fructosidases can hydrolyse the terminal fructose residue of raffinose, leaving melibiose unfermented (Walker 1998), which may also contribute to the rise in fructose.

1.7. Water relations in dough above freezing

Water is essential to the formation of dough, and the amount of water exerts a strong influence on mixing characteristics and bread quality (Eliasson and Larsson 1993). It has long been recognised that flours from different wheat varieties differ in water uptake (and hence breadmaking) characteristics. A number of authors have sought correlations between flour composition parameters such as protein or starch content and water absorption capacity (Greer and Stewart 1959; Farrand 1969; Shogren et al. 1987; Morrison et al. 1989; Finney and Bains 1999; Berton et al. 2002).

Correlations have been good in several cases, and highlight the fact that pentosans and damaged starch granules take up a lot of water (Shogren et al. 1987; Berton et al. 2002; Roman-Gutierrez et al. 2002a).

Early calculations using published water-holding capacities of flour components (Larsen 1964; Bushuk 1966) suggested that much of the water in dough was associated with starch, gluten and pentosans, though little was known about the nature of such associations at the time. Although these calculations neglected interactions among components, they served to illustrate the scarcity of water in dough (Bushuk 1966) and brought to light the relevance of water uptake thermodynamics, i.e. the energetic states in which water may exist, and kinetics, i.e. how fast it moves between those states.

1.7.1 Water uptake kinetics

Relatively little is known about how water distribution in dough changes with mixing and fermentation. This is probably a result of the highly dynamic nature of dough, in which physical properties change on a time scale of minutes. Dough undergoes rheological changes during fermentation (Lee et al. 2004), which indicates that dough structure is changing, but the involvement of water is not well-understood.

When flour is mixed with water, gluten proteins swell and absorb water. The mixing action stretches and aligns peptide chains, which associate with each other via covalent disulphide bonds, noncovalent hydrogen bonds and hydrophobic interactions (Bloksma 1990b; Potus et al. 1992; Tolstoguzov 1997). Water-soluble proteins, pentosans and low molecular weight solutes dissolve to form a mixed protein-polysaccharide aqueous phase incompatible with the hydrated gluten gel (Bloksma 1990b; Eliasson and Larsson 1993; Tolstoguzov 1997). Water forms the continuous medium of both phases (Tolstoguzov 1997).

The equilibrium between phases is influenced by the balance of hydrophilic and hydrophobic forces in the dough, for example the hydrophobic interactions contribute to the structure of gluten (Bloksma 1990b; Potus et al. 1992). Dissolved solutes influence the phase equilibrium by their effect on the properties of the aqueous phase. Salt (NaCl) dissociates into Na^+ and Cl^- ions, which reduce repulsive forces between charged peptides through a shielding effect, but also promote hydrophobic interactions through their effect on water structure (Eliasson and Larsson 1993).

Some reports indicate that gluten takes up more water as it is developed (Larsson and Eliasson 1996b; Bot and De Bruijne 2003) and that average water mobility falls (Leung et al. 1979; Ruan and Chen 1998) but others have suggested that water is released from gluten as a result of work input (Webb et al. 1970; Daniels 1975; Larsson and Eliasson 1996a). In one study the 'average binding energy' of water increased with up to 8 minutes' mixing then declined with 8-20 minutes' mixing (Bushuk and Mehrotra 1977a).

Two early studies found that the rate of water uptake by flours varied inversely with protein content, from which the authors concluded that starch granules took up water faster than gluten (Larsen 1964; Udani et al. 1969). A more recent study with a controlled atmosphere microbalance found no difference between moisture uptake kinetics of gluten and starch exposed to a step increase in relative vapour pressure from 66% to 76% (Roman-Gutierrez et al. 2002a). The authors noted that pentosans had particularly high water sorption capacity, and insoluble pentosans took up moisture rapidly.

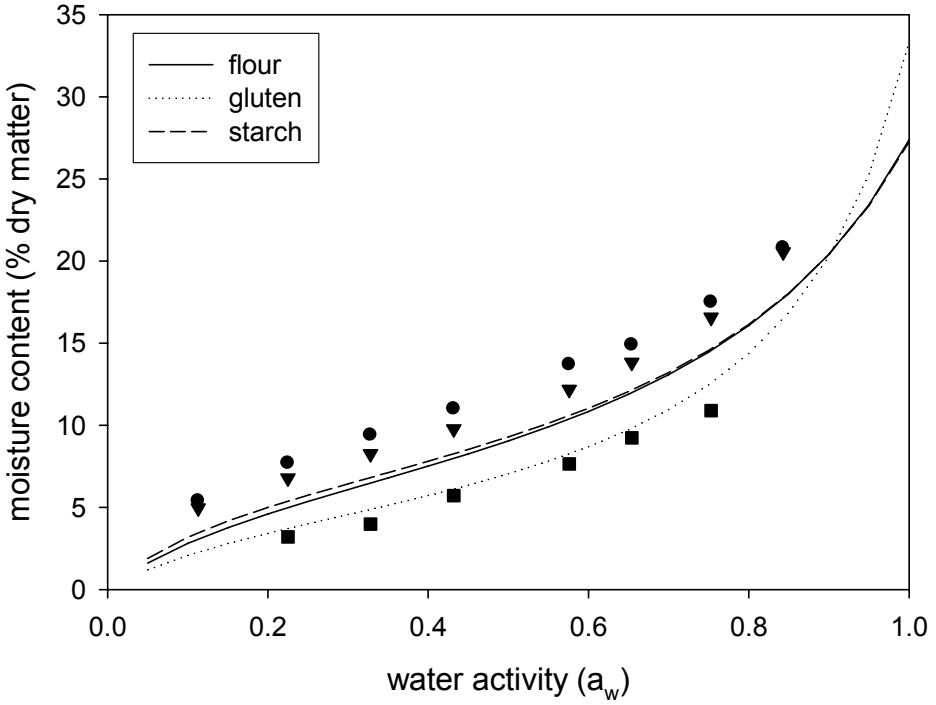
1.7.2 Water uptake thermodynamics

Dipole-ion and dipole-dipole interactions with solutes reduce the mobility of water molecules in a solution relative to pure water. This phenomenon is widely expressed as water activity (a_w) which is defined as the fugacity or 'escaping tendency' of water in a test substance relative to pure water. For practical purposes a_w is well-approximated by the partial pressure of water vapour above a substance divided by the partial pressure over pure water at the same temperature (Fennema 1996), also known as relative vapour pressure (RVP) or relative humidity (RH). Plots of a_w vs. moisture content known as moisture sorption isotherms are useful for describing water sorption behaviour of foods.

Moisture sorption isotherms have been determined for flour (Bushuk and Winkler 1957; Lee 1970; Roman-Gutierrez et al. 2002a), starch (Bushuk and Winkler 1957; Roman-

Gutierrez et al. 2002a), gluten (Bushuk and Winkler 1957; Cherian and Chinachoti 1996; Roman-Gutierrez et al. 2002a), pentosans (Roman-Gutierrez et al. 2002a) and dough (Lind and Rask 1991; Laaksonen and Roos 2000, 2003).

Figure 1.7. Moisture sorption isotherms at 25˚C for major dough components.



Lines are GAB equation isotherms from parameters reported by Roman-Gutierrez et al. (2002a). Squares show desorption data from dough (Laaksonen and Roos 2003), circles are adsorption data from freeze-dried dough (Laaksonen and Roos 2000). Triangles are predictions from the Oswin model fitted to dough desorption data (Lind and Rask 1991).

Isotherms display the classical sigmoidal form common to many foods and biopolymers (Figure 1.7) and show adsorption-desorption hysteresis (Bushuk and Winkler 1957). They are well-fitted by the GAB equation (Cherian and Chinachoti 1996; Laaksonen and Roos 2000; Roman-Gutierrez et al. 2002a), but the BET (Bushuk and Winkler 1957; Laaksonen and Roos 2000) and Oswin models (Lind and Rask 1991) have also been successfully applied across parts of the water activity spectrum. Moisture sorption isotherms are shifted to lower a_w by the addition of salt or sugar (Sa and Sereno 1994; Laaksonen and Labuza 2001).

Flour shows very similar sorption behaviour to starch (Bushuk and Winkler 1957; Roman-Gutierrez et al. 2002a), which is unsurprising considering that it comprises 75-

90% of flour dry matter. Gluten absorbs less water than flour or starch (Roman-Gutierrez et al. 2002a).

Both the amount of water adsorbed by flour (Bushuk and Winkler 1957) and the rate of absorption (Udani et al. 1969) are superimposable for different size fractions of a given flour. This has been interpreted to mean that water adsorption occurs at specific sites on flour particles and is independent of surface area (Udani et al. 1969; Lee 1970).

It has recently been suggested that water in dough is present in discrete 'microstructural domains' with distinct local conditions (Kou et al. 2002). The collective contribution of domain properties to macroscopic dough properties is represented in probability distributions. The location and spread of peaks in relaxation time probability distributions indicate the mobility and heterogeneity (respectively) of water molecule populations in dough (Ruan and Chen 1998; Ruan et al. 1999).

Kinetic studies (Kou et al. 2002) suggest that viscosity in microstructural domains of dough is decreased by an increase in temperature or moisture content, and that heterogeneity is increased at higher temperature or lower moisture content.

NMR studies of doughs (Leung et al. 1983) or gluten (Cherian and Chinachoti 1996) hydrated with ^{2}H- or ^{17}O-rich water have not been able to resolve multiple water molecule populations or distinguish between flours of different protein content, but a general increase in water mobility with increasing moisture content has been demonstrated.

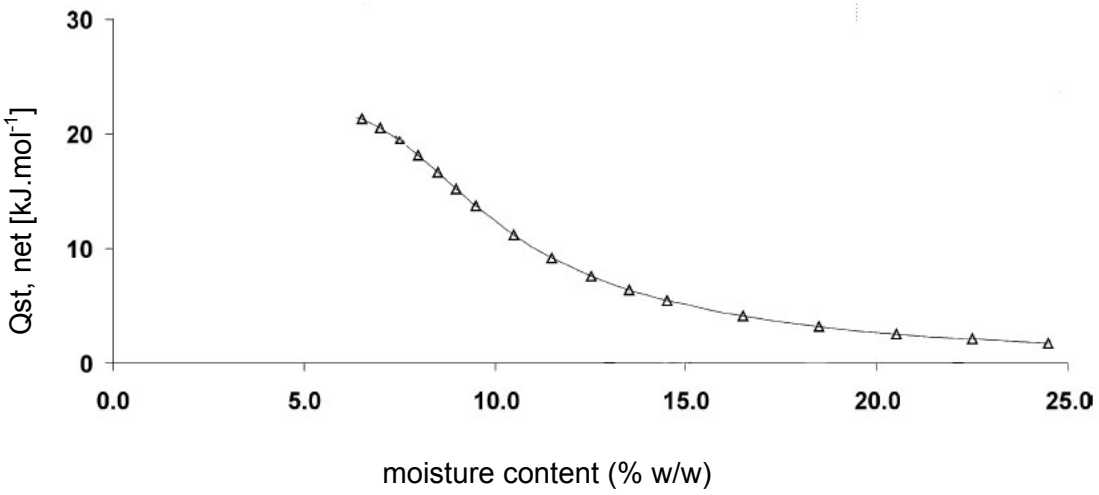
1.7.3 Hydration sequence in flour-water mixtures

This section describes four loosely-delineated stages involved in hydrating flour and the formation of a dough. The water content of flour depends on milling and storage conditions, but is typically close to 14%. Water activity at this moisture content is 0.7-0.8 (Roman-Gutierrez et al. 2002a).

1.7.3.1. Stage I

Below 20-25 grams H_2O per 100g dry matter (g H_2O.(100g DM)$^{-1}$) or a_w ~0.8 water interacts strongly with polymers in flour (proteins, starch, pentosans), and has physical properties markedly different from pure water. The latent heat of adsorption is greater than the latent heat of condensation, i.e. the net isoteric heat of adsorption (Q_{st} in Figure 1.8) is positive (Bushuk and Winkler 1957; Ruckold et al. 2003).

Figure 1.8. Net isoteric heat of sorption ($Q_{st, net}$) for water adsorbing to flour.



Q_{st} is the difference between the latent heat of adsorption and the latent heat of condensation of water. Reproduced from (Ruckold et al. 2003).

Water at this level will not freeze at -50˚C (Toledo et al. 1968) and enzyme activity is inhibited by the scarcity of available water (Lee 1970). Below 23 g H_2O.(100g DM)$^{-1}$ two proton populations with low and intermediate mobility are observed in NMR experiments (Ruan et al. 1999). Flour-water mixtures will not support the force of their own weight below 18-23 g H_2O.(100g DM)$^{-1}$ (Davies et al. 1969)

1.7.3.2. Stage II

Between 25 and 33 g H_2O.(100g DM)$^{-1}$ (a_w 0.8-0.9) a flour-water mixture will form a dough capable of supporting its own weight (Davies et al. 1969) and the extractability of flour lipids increases dramatically (Wood et al. 1972; Daniels 1975). Enzymes become active but activity is limited by the availability of water (Lee 1970).

The isoteric heat of adsorption approaches that of pure water at 20-30 g H_2O.(100g DM)$^{-1}$, indicating capillary condensation (Bushuk and Winkler 1957; Ruckold et al. 2003). Moisture sorption isotherms curve sharply upwards in this region (Bushuk and Winkler 1957; Roman-Gutierrez et al. 2002a).

Early wideline ^{1}H NMR studies reported 'bound' water capacities at the upper end of this region for flour (32.6 g H_2O.(100g DM)$^{-1}$), starch (29.6 g H_2O.(100g DM)$^{-1}$) and gluten (36.9 g H_2O.(100g DM)$^{-1}$) (Leung and Steinberg 1979). NMR signals show characteristics of freezable water above 24.6 g H_2O.(100g DM)$^{-1}$ (Toledo et al. 1968).

Recent NMR studies have resolved proton relaxations in dough into 3 components or continuous spectra (Ruan and Chen 1998; Ruan et al. 1999; Kou et al. 2002; Esselink et al. 2003). Increasing moisture content from 23 to 35 g H_2O.(100g DM)$^{-1}$ leads to (a) the disappearance of low-mobility proton population (b) enlargement of the intermediate-mobility population, which shifts to slightly lower mobility and (c) appearance of one or more high-mobility populations (Ruan and Chen 1998; Ruan et al. 1999).

The three populations are thought to correspond with 'constitutional' water molecules intimately involved in the internal structure of macromolecules (Fennema 1996), water molecules rapidly sampling hydration sites on the surface of gluten and starch (Esselink et al. 2003) and water that associates weakly with the dough matrix (Ruan and Chen 1998; Esselink et al. 2003).

Caution should be exercised in assigning the low-mobility proton population to low-mobility water molecules, since proton exchange between water molecules and biopolymer protons is thought to occur in some systems (Hills et al. 1990; Hills et al. 1991; Cherian and Chinachoti 1996; Vackier et al. 1999).

1.7.3.3. Stage III

At 33-50 g H_2O.(100g DM)$^{-1}$ (a_w 0.95) enzyme activity is no longer limited by water availability and becomes dependent on the concentration of enzyme (Lee 1970). The limiting water content for concentration-dependent enzyme activity is higher in doughs with added sucrose or glucose, but occurs at the same water activity (Lee 1970).

In NMR experiments increasing moisture between 35 and 45 g H_2O.(100g DM)$^{-1}$ produced little change in size of the intermediate-mobility proton fraction, which appeared to be saturated (Ruan and Chen 1998; Ruan et al. 1999). The high-mobility fraction continued to grow, became more disperse and shifted to slightly higher mobility (Ruan and Chen 1998; Ruan et al. 1999).

DTA studies (Davies and Webb 1969; Bushuk and Mehrotra 1977b) and DSC experiments (Roman-Gutierrez et al. 2002b) reported the appearance of freezable water in doughs with more than 30-33 g H_2O.(100g DM)$^{-1}$**.** These findings should be interpreted cautiously in light of objections raised by Hatley et al. (1991) to such methodologies.

Davies and Webb (1969) concluded that all water above 33 g H_2O.(100g DM)$^{-1}$ was freezable at -50˚C, in agreement with the NMR study of Toledo et al. (1968). Others

calculated that only 65-83% was freezable, which was thought to indicate that water plasticised and swelled flour particles in a way that favoured increased interaction with water (Bushuk and Mehrotra 1977b; Roman-Gutierrez et al. 2002b).

1.7.3.4. Stage IV

Above 50 g H_2O.(100g DM)$^{-1}$ a dough will retain gas (MacRitchie 1976) and can be centrifugally separated into phases (MacRitchie 1976; Larsson and Eliasson 1996a). Doughs will conduct charge, which indicates the appearance of a continuous liquid phase (MacRitchie 1976). Webb et al. (1970) measured extensibility as a function of moisture content at a range of work inputs, and extrapolation indicated incipient extensibility at 50 g H_2O.(100g DM)$^{-1}$.

1.7.4 Summary

The evidence above paints a picture of flour hydration in 4 loosely-delineated stages:

- Up to 20-25 g H_2O.(100g DM)$^{-1}$or a_w 0.8-0.9, water molecules interact extensively with flour components, and therefore are difficult to remove (high Q_{st}), display low mobility and fugacity and will not freeze above –50˚C.
- With 25-33 g H_2O.(100g DM)$^{-1}$ or a_w 0.95 the isoteric heat of sorption approaches the heat of condensation of pure liquid water. Water mobility is intermediate between biopolymer hydration water and bulk water, and water won't crystallise on an experimental time scale. In this range doughs have sufficient coherence to support their own weight and enzymes become partially active.
- Additional water in the range 30-50 g H_2O.(100g DM)$^{-1}$ plasticises flour macromolecules and exposes more hydrophilic groups to interactions with water molecules. Water becomes freezable, enzymes are fully active and yeast activity is no longer limited by water availability.
- Above 50 g H_2O.(100g DM)$^{-1}$ dough is extensible and capable of retaining gas and conducting charge.

1.8. Water relations in dough below freezing

1.8.1 The formation and stability of ice crystals

The removal of sensible heat from a food results in a drop in temperature without phase change. At the freezing point the water vapour pressure of the liquid phase becomes equal to that of ice at the same temperature, but an energetic barrier to crystallisation prevents spontaneous ice formation and the aqueous phase undercools at first.

With a few degrees of undercooling, crystallisation of water on solid particles or surfaces becomes energetically favourable and ice nuclei form where suitable conditions exist, accompanied by the release of latent heat. Nucleation is inherently unpredictable, and may occur at a number of sites simultaneously. Once nucleation has occurred, crystals can grow with only slight undercooling.

When undercooling is extensive due to rapid heat removal, many nuclei are formed and the frozen food contains many small ice crystals. Slower cooling allows each nucleus to grow more extensively, producing fewer, larger ice crystals.

The water-ice phase change is accompanied by a 9% increase in specific volume. Thermal diffusivity rises almost 9-fold and conduction of heat through the frozen outer part of the dough is rapid. As water is deposited in nearly pure ice crystals, the concentration of solutes in the remaining unfrozen phase rises. The equilibrium freezing temperature is depressed further, and viscosity rises due to freeze-concentration and falling temperature.

Freeze-concentration of solutes can accelerate some chemical reactions, but retardation by low temperature and high viscosity counteracts this effect. Hence some reaction rates go through a maximum as temperature declines below the initial freezing point (Fennema 1973).

Growth of ice crystals requires removal of latent heat, diffusion of water molecules to the ice-solution interface and rejection of solute molecules away from the interface. When intermediate and high-water foods are frozen at moderate rates, propagation of the freezing front is initially limited by the transfer of latent heat. Water molecules are small and diffuse rapidly, but if rejection of solutes is inhibited by high viscosity or

obstruction by macromolecules, ice propagation becomes mass-transfer limited. The region ahead of the freezing front undercools and departs from equilibrium.

During frozen storage the total amount of ice will not change unless ice formation was kinetically inhibited during freezing, however ice crystals may undergo changes in shape and size. Migratory recrystallisation, also called 'grain growth' and 'Ostwald ripening,' refers to the tendency for large crystals to grow at the expense of small crystals (Walstra 1996; Jeremiah 1996). At constant temperature this is driven by the vapour pressure difference between small and large crystals, which results from different radii of curvature. The dependence of vapour pressure on curvature is expressed in the Kelvin equation (equation 1.15) (Franks 1985).

Small crystals also have a higher specific interfacial energy than larger ones, due to the smaller radius of curvature (Pham and Mawson 1997). The rate at which small crystals sublime and large crystals grow under isothermal conditions depends on the viscosity of the aqueous phase (Franks 1985) and the activation energy for molecules to move to or from the crystal surface (Pham and Mawson 1997).

$$RT \ln \frac{p}{p^*} = \frac{2\gamma M}{\rho r} \qquad 1.15$$

R	universal gas constant	[N.m.K^{-1}.mol^{-1}]
T	absolute temperature	[K]
p	vapour pressure over a curved surface	[N.m^{-2}]
p^*	vapour pressure over a plane surface	[N.m^{-2}]
M	molar mass	[g.mol^{-1}]
ρ	density	[g.m^{-3}]
γ	surface tension	[N.m^{-1}]
r	surface radius of curvature	[m]

The enlarging of large ice crystals at the expense of smaller ones is accelerated by temperature fluctuations, which cause the smallest crystals to melt completely as temperature rises. On cooling, water is deposited on the surface of surviving larger

crystals in preference to formation of new nuclei (Fennema and Powrie 1964; Franks 1985; Pham and Mawson 1997). Temperature gradients within a food lead to vapour pressure gradients, resulting in moisture migration (Reid 1997).

Thawing is not the reverse of freezing because of the difference in heat transfer characteristics of frozen and unfrozen substance. Ice is a better conductor of heat than water, and at the start of thawing heat is transferred rapidly through the frozen substance. Internal temperature rises rapidly then remains close to melting temperature as heat transfer is slowed by the absorption of latent heat at the melting front and the insulating action of an exterior melted region. Recrystallisation and chemical reactions can occur rapidly while the interior remains just below the melting temperature.

1.8.2 Nonequilibrium freezing and the glass transition

As temperature is reduced during freezing and concentration in the unfrozen phase increases with ice formation, viscosity increases (Reid et al. 1994). If viscosity is sufficiently high crystallisation of solutes at their equilibrium eutectic temperatures is prevented, and the supersaturated unfrozen solution undercools further (Reid et al. 1994). At some point ice formation will cease and an abrupt rise in viscosity over a narrow temperature range is seen, accompanied by large changes in heat capacity, volume expansion coefficient and dielectric properties (White and Cakebread 1966; Champion et al. 2000). The undercooled solution becomes 'rubbery' then eventually 'glassy' as viscosity approaches 10^{10} – 10^{12} Pa.s (Levine and Slade 1988).

Glass transition phenomena in foods have been reviewed extensively (White and Cakebread 1966; Blanshard and Lillford 1993; Reid et al. 1994; Roos et al. 1996; Champion et al. 2000; Le Meste et al. 2002). The glass transition temperature (T_g) is not sharply defined owing to the kinetic nature of the transition, and the frequency of measurement therefore determines the range over which it is seen (Reid et al. 1994).

Considerable controversy surrounds the experimental observation of the glass transition, and reports of T_g vary depending on the method of observation and the interpretation of data (Simatos and Blond 1993; Reid et al. 1994). The glass transition may be obscured by the ice-water phase transition, and glass transitions in foods are often spread over a wide temperature range because of unresolved transitions of different components (Le Meste et al. 2002).

The high viscosity in a glass reflects low molecular mobility, and the rate of diffusion-limited processes such as enzyme-catalysed reactions is highly temperature-dependent in the region 10-100˚C above the glass transition temperature (Fennema 1996). Reactions involved in deterioration of food may be inhibited if a glassy state can be attained, hence the glass transition temperature is of practical significance in the formulation and processing of foods (Lim and Reid 1991; Simatos and Blond 1993).

Glass transition temperature is strongly dependent on molecular weight, and glassy polymer matrices are plasticised by small molecules, most commonly water in food systems (Fennema 1996; Champion et al. 2000; Le Meste et al. 2002).

In frozen foods T_g of the unfrozen phase is controlled by the amount of ice present. If the food is cooled sufficiently slowly that the unfrozen phase is in equilibrium with ice, it will be maximally freeze-concentrated and its glass transition temperature is denoted T_g' (Champion et al. 2000). A more realistic scenario is that heat removal outstrips ice formation and the food undercools, i.e. the unfrozen phase contains more than the equilibrium amount of water and T_g is below T_g'.

1.8.3 Dough freezing and frozen storage

1.8.3.1. Freezing

The temperature range over which water in dough will crystallise depends on the amount of material dissolved in the aqueous phase, especially low molecular weight solutes such as salt and sugar. Initial freezing points between -0.45 and -2.81 (Skovholt and Bailey 1935) and final melting point between -0.27 and -2.81 (Vail and Bailey 1940) have been reported, depending on the amount of sugar in the dough formulation. Laaksonen and Roos (2001a) found that adding 6% sucrose depressed initial melting point of frozen dough by 3˚C, and 1.5% NaCl produced a 6-9˚C melting point depression.

Freezing point measurement is complicated by the unpredictability of nucleation, and it is difficult to control or predict the level of undercooling in dough. Initial melting points of frozen dough are more consistent, but depend on the way in which dough is frozen. Holding dough at sub-freezing temperature prior to measurement (annealing) depresses initial melting point from between -10 and -12˚C (Räsänen et al. 1998; Stecchini et al. 2002) to between -18 and -20˚C (Lind 1988, 1991b; Laaksonen and Roos 2000, 2001b).

When oscillatory measurements are used the temperature at which the melting transition is observed depends on measuring frequency. For example initial melting points measured with dielectric thermal analysis (DETA) vary between -3 and -14˚C for dough frozen without annealing (Räsänen et al. 1998) and 0 to -24˚C with annealed dough (Laaksonen and Roos 2000, 2001a).

Ice content increases rapidly once freezing is initiated, with 52-57% of the total water in dough frozen at –10˚C (Lind 1991b; Räsänen 1998; Lee et al. 2002). Further cooling produces little extra ice: at –20˚C slightly more than 60% of the water is frozen (Lind 1991b; Lee et al. 2002).

Lind (1991b) and Baik et al. (2001) have reviewed the measurement and modelling of thermal properties of bakery products such as specific heat, thermal conductivity and thermal diffusivity. Thermal conductivity of bakery products has been mathematically modelled by Sablani et al. (2002).

1.8.3.2. Glass transition properties

The DMTA glass transition occurs in dough between -10 and -44˚C and is sensitive to flour composition and the amount of water (Räsänen et al. 1998; Laaksonen and Roos 2000; Laaksonen and Roos 2001a). T_g sometimes increases during frozen storage (Räsänen et al. 1998), but this is not seen in reduced-water doughs (Räsänen et al. 1998) or where samples are annealed prior to analysis (Laaksonen and Roos 2000).

Adding salt or sugar to a dough at constant water activity increases water sorption and depresses T_g (Laaksonen and Roos 2003, Laaksonen and Labuza 2001), in accordance with polymer science principles that water can plasticise polymer matrices (Levine and Slade 1991). At constant water content added sugar raises T_g (Laaksonen and Roos 2001a, b) and salt raises DMA T_g (Laaksonen and Roos 2001b), but depresses DEA T_g (Laaksonen and Roos 2001a).

Glass transition curves have been published for gluten (Hoseney et al. 1986; Fujio and Lim 1989; Cherian and Chinachoti 1996), glutenin (Kokini et al. 1994), gliadin (De Graaf et al. 1993; Kokini et al. 1994) and dough centrifugate (Stecchini et al. 2002).

Thermal features associated with the glass transition have not been observed in dough or gluten using differential scanning calorimetry, which may be due to insufficient sensitivity (Laaksonen and Roos 2000; Stecchini et al. 2002).

It is not clear which component or phase of dough undergoes a glass transition in the above reports, but T_gs are almost all below normal freezer temperature (-18 or -20˚C), indicating that frozen dough is in a rubbery state during storage.

1.8.3.3. Frozen storage

There is evidence for migration of water from the dough matrix into ice crystals during the first few weeks of frozen storage.

When frozen dough is thawed and subjected to a freeze-thaw cycle in a DSC pan, the melting enthalpy goes up with longer frozen storage time, which has been interpreted to indicate an increase in freezable water (Lu and Grant 1999; Sharadanant and Khan 2003; Bhattacharya et al. 2003). However when dough samples are transferred directly from frozen storage to the DSC and thawed once, there is little change in the melting enthalpy with frozen storage time (Bot 2003; Baier-Schenk et al. 2005), which suggests that the amount of ice is constant. This does not preclude changes to the shape or size of crystals, i.e. recrystallisation (Baier-Schenk et al. 2005).

Longer frozen storage also leads to higher water mobility in thawed dough (Esselink et al. 2003) and an increase in the amount of liquid phase that separates from centrifuged dough (Räsänen et al. 1998). The DMTA glass transition temperature increases during the first two weeks of frozen storage (Räsänen et al. 1998), consistent with removal of plasticizing water molecules from structural elements of the dough matrix.

Scanning electron microscope (SEM) images show the growth of ice crystals during frozen storage (Zounis et al. 2002a, 2002b; Baier-Schenk et al. 2005) and highlight large ice crystals on the inside surface of gas cells (Esselink et al. 2003; Baier-Schenk et al. 2005). Concomitant with ice crystal growth is visible deterioration of gluten (Berglund et al. 1991; Varriano-Marston et al. 1980) and infra-red spectroscopy evidence points to gluten dehydration during extended frozen storage (Esselink et al. 2003).

1.9. Biological stresses in frozen dough

1.9.1 Nature and origin of freezing stresses

Direct observation of yeast cells in frozen dough is complicated by the dense dough matrix, but the general principles underlying the response of biological cells to freezing should apply.

Ice formation may be initiated inside or outside a cell, but typically more effective nucleating agents are present outside cells and ice forms externally at first (Mazur 1966, 1970). Crystallisation of water concentrates the extracellular solution and produces an osmotic differential between the external solution and unfrozen cytoplasm (Schwartz and Diller 1983). The differential is resolved by either transfer of water out of the cell or internal nucleation, hence cells either dehydrate or freeze intracellularly (Mazur 1966, 1970; Reid 1993; Erickson and Hung 1997).

Which possibility eventuates is determined by the relationship between cooling velocity and the capacity for water transfer across the cell membrane (Levin 1979; Schwartz and Diller 1983; Reid 1993). Slow cooling and high permeability to water favour equilibration by dehydration (Mazur 1970). In fast cooling or where water permeability is low, the capability to export water may be outstripped, and the cytoplasm becomes sufficiently undercooled that intracellular ice forms (Levin 1979). Mechanical stresses resulting from impingement of rigid ice crystals and shrinkage of dehydrated cells can damage cellular structures (Blakebrough 1967; Reid 1993).

Freeze-concentration of intracellular and extracellular solutes gives rise to detrimental ‘solution effects’ (Mazur 1970). Solutes may precipitate as their eutectic concentrations are exceeded, causing changes in the ionic and chemical environment, e.g. pH shifts (Van den Berg 1959, 1968), that are disruptive to the functioning of enzymes (Mazur 1966; Reid 1993). Levitt (1962) proposed that proteins brought into close vicinity in the concentrated cytoplasm could form covalent disulphide bonds with each other, causing them to lose their activity on thawing. However freezing injury is more often attributed to membrane damage and disruption of membrane-mediated processes rather than denaturation of soluble proteins (Mazur 1970).

Lovelock (1953) suggested that exposure to high salt concentrations damaged the cell membrane of red blood cells, and the enhanced permeability of the membrane to ions led to uncontrolled ingress of water and cell lysis on thawing.

Survival of yeast cells frozen in water shows a maximum when cooling velocity is around 10˚C.min^{-1} (Araki and Nei 1962; Mazur and Schmidt 1968). Slower cooling may result in extended exposure to 'solution effects' associated with cryoconcentration of the cytoplasm, whereas faster cooling may lead to intracellular freezing (Mazur and Schmidt 1968; Mazur 1970).

When cells are cooled very rapidly to low temperature, small intracellular ice crystals are prevented from growing by the rise in viscosity of the cytoplasm. However they may enlarge during slow warming and disrupt cellular structures, particularly membranes (Mazur 1966, 1970; Mazur and Schmidt 1968). Rapidly-cooled yeast cells are considerably more sensitive to warming rate than slowly-cooled cells, and show good survival only when warming is rapid (Mazur and Schmidt 1968).

Mathematical modelling studies (Mazur 1970; Levin 1979) and direct observation of cells undergoing freezing (Schwartz and Diller 1983) indicate that cooling velocities in excess of 9-10˚C.min^{-1} are required to produce intracellular freezing in yeast cells. At above-freezing temperatures yeast cells can dehydrate in a matter seconds in response to moderate osmotic shocks (Berner and Gervais 1994; Martinez-DeMaranon et al. 1997) and they will survive large shifts in osmotic pressures provided the increase is not too rapid (Gervais and Marechal 1994; Marechal et al. 1995).

Considering that freezing rates slower than 2˚C.min^{-1} are typical in frozen dough manufacture (Hsu et al., 1979b; Havet et al., 2000), it is likely that yeast cells remain close to osmotic equilibrium with the dough aqueous phase, at least during the initial stages of freezing. The deleterious effects of rapid cryogenic freezing (Neyreneuf and Delpuech 1993; Masovic and Jankovic 2002) and freezing to -40˚C or colder (Hsu et al. 1979b) may be attributable to intracellular freezing.

1.9.2 Cellular stress responses

Freezing and thawing exposes yeast cells to large changes in external osmotic pressure, and cellular responses that mitigate the effects of osmotic stress often confer tolerance to freezing as well.

When external osmotic pressure increases, yeast cells shrink quite rapidly, reflecting the high permeability of the cell membrane to water (Blomberg and Adler 1992; Gervais and Marechal 1994; Martinez-DeMaranon et al. 1997; Beney et al. 2000). Tanghe et al. (2002) found that the freezing tolerance of freeze-sensitive strains could be improved by over-expression of genes coding for aquaporins: trans-membrane proteins

that facilitate water transport. Although aquaporin overexpression improved tolerance to rapid freezing under laboratory conditions, this did not translate into an improvement under industrial conditions of large doughs and slow freezing (Tanghe et al. 2004).

Slower variations in osmotic pressure allow cells to actively regulate their intracellular osmotic potential by manipulating the concentration of small organic molecules known as compatible solutes. These typically exhibit high solubility in the cytoplasm and slow passive diffusion through the cell membrane, and they must be innocuous to proteins at high concentration (Blomberg and Adler 1992; Gutierrez et al. 1995).

Compatible solutes counteract the deleterious effects of freezing in several ways (Mazur 1966, 1976):

- Colligative depression of cytoplasm water activity, counteracting the tendency to dehydrate and lessening the rise in electrolyte concentration.
- Direct interaction with organelles or macromolecules to maintain structure and function
- Inhibiting the nucleation or growth of intracellular ice crystals

In yeasts, the most important compatible solutes are the polyhydroxy alcohols and sugars, particularly glycerol and trehalose (Blomberg and Adler 1992; Walker 1998).

1.9.2.1. Glycerol

When yeasts ferment glucose to ethanol, production of glycerol by reduction of dihydroxy acetone phosphate is required to maintain the redox balance (Blomberg and Adler 1992; Walker 1998). The intracellular concentration of glycerol is high in cells growing under osmotically stressful conditions (Panchal and Stewart 1979; Blomberg and Adler 1992; Myers et al. 1997; Attfield and Kletsas 2000), and its protective effect during freezing of micro-organisms is well-known (Mazur 1970).

This effect has been attributed to dilution of electrolytes in the unfrozen solution. Mazur (1976) illustrated this as follows: when a 0.3 osmolal NaCl solution is frozen to -10˚C, the unfrozen phase contains 5.4 osmolal NaCl. When a solution containing 0.3 osmolal NaCl and 1 osmolal glycerol is frozen to the same temperature, total osmolality is still 5.4 osmolal, but the unfrozen phase consists of 4.14 osmolal glycerol and only 1.26 osmolal NaCl. The protective effect of glycerol is lost when freezing is very rapid (Lewis et al. 1993).

Myers et al. (1997) examined the impact of osmotic pressure on gas production of 14 yeast strains in a liquid broth designed to model conditions in dough. Although freezing tolerance was not examined, good performance at high osmotic pressure was associated with the ability to synthesise and retain high levels of glycerol. Using the same medium, Attfield and Kletsas (2000) reported that strains accumulating the most glycerol also produced the most ethanol in high-sugar medium. Loading of yeast with glycerol prior to inoculation in doughs with high levels of added sugar improves gassing power, particularly in the initial hour of fermentation (Myers et al. 1998).

There has been relatively little interest in glycerol in the frozen dough literature. In frozen dough, treatment of yeast with glycerol retards the increase in proofing time seen during frozen storage, particularly when yeast is refrigerated for an extended duration prior to use (Myers and Attfield 1999). Direct addition of glycerol to dough has also been reported to slow the loss of gassing power during frozen storage (Bender and Lamb 1977).

1.9.2.2. Trehalose

The disaccharide α-α-trehalose (α-D-glucopyranosyl (1→1) α-D-glucopyranoside) has received intense interest in the frozen dough industry recently. The physical properties of trehalose are reviewed in Richards and Dexter (2001).

Trehalose stabilises biological and synthetic membranes against phase transition during dehydration (Crowe and Crowe 1982; Clegg et al. 1982; Crowe et al. 1992, 1996) and it preserves the native state of proteins in low-water conditions (Arakawa and Timasheff 1982; Crowe et al. 1991; Xie and Timasheff 1997). Although trehalose appears not to be involved in osmoregulation during steady state growth, it is accumulated to high levels in the stationary phase (Blomberg and Adler 1992; Lewis et al. 1993).

The protective effect of trehalose at low water content is due to either replacement of structural water molecules at specific sites (Clegg et al. 1982; Carpenter and Crowe 1989; Sano et al. 1999), vitrification of trehalose molecules interspersed with membrane lipids (Crowe et al. 1992; Crowe et al. 1996) or a combination of both. The mobility of water molecules is reduced in the vicinity of a trehalose molecule, but the significance of this in biological systems is not clear (Sakurai et al. 1995, 1997; Qiang et al. 1996; Sano et al. 1999).

Trehalose accumulation in yeast has been found to correlate with resistance to heat stress (Attfield et al. 1992; Eleutherio et al. 1993; Van Dijck et al. 1995; Pereira et al. 2001) and exposure to ethanol (D'Amore et al. 1991; Mansure et al. 1994; Pereira et al. 2001), hydrogen peroxide, salt or acetic acid (Lewis et al. 1997). Trehalose reduces ethanol-induced leakage from synthetic liposomes that mimic the yeast cell membrane, and a similar effect is seen in yeast cells in some cases (Mansure et al. 1994).

Given the evidence that trehalose can counteract some of the stresses encountered in freezing (e.g. dehydration, low-temperature-induced membrane phase transitions, cryoconcentration of ethanol), it might be expected that good trehalose accumulation would be associated with tolerance to freezing and superior frozen dough performance. Indeed, this has been observed in some cases, and some authors (e.g. Van der Plaat (1988), Casey and Foy (1995)) advocate for a central role of trehalose in determining the frozen dough performance of yeast. However, the experimental evidence is by no means clear-cut.

Hino et al. (1990) reported that trehalose reserves were catabolised rapidly in the first 15 minutes of fermentation, leaving only a low basal level with longer fermentation. Strains that retained higher basal trehalose generally showed greater viability after freezing in liquid medium and better performance in frozen doughs pre-fermented up to 180 minutes.

Tanaka et al. (1980) examined the impact of storage at 5˚C on the frozen dough performance of seven commercial compressed yeast products. The trehalose content of compressed yeast fell consistently during storage. The gassing power of unfermented frozen doughs declined as yeast was stored for longer before use, but the gassing power of pre-fermented frozen doughs improved with longer storage.

Several authors have sought a relationship between trehalose level and cryotolerance or the retention of gassing power in frozen yeasted doughs (Oda et al. 1986; Gelinas et al. 1989; Meric et al. 1995; Almeida and Pais 1996). Correlations have been at only moderately strong at best, but mostly nonsignificant. Lewis et al. (1997) suggested several reasons for the lack of strong linear correlations, including:

- the relation may not be strictly linear.
- the effect of trehalose may vary with growth phase.
- there may be a ‘threshold’ value of trehalose for stress tolerance, a view also advocated by Gadd et al. (1987) and Meric et al. (1995).

- physiological and genetic variation between the unrelated strains may obscure any trehalose effect, which might be more evident in closely related strains.

Meric et al. (1995) were the first workers to directly extract trehalose from dough, and they examined the relation between trehalose content and gassing power in doughs fermented up to 180 minutes prior to frozen storage. Trehalose always declined during mixing and fermentation, but to an extent dependent on temperature and strain. There was no direct correlation between trehalose and the retention of gassing power in frozen fermented doughs, but it was evident that with less than 4-5 g trehalose per 100 g yeast dry matter, resistance to freezing was seriously compromised. Freeze-thaw resistance increased with increasing trehalose content up to 5%, and further trehalose did not improve freeze-thaw resistance.

1.9.3 Impact of fermentation wastes

When yeast is mixed with other dough ingredients, cells are hydrated and exposed to nutrients. They begin actively metabolising and adapt to extrinsic conditions, a process that continues during fermentation. One hypothesis to explain the effect of pre-fermentation on frozen dough stability is that adapted and fully active cells are more vulnerable to freezing damage than resting cells (Tanaka and Miyatake 1975; Hsu et al. 1979a; Neyreneuf and Van der Plaat 1991).

This is certainly the case in broth culture, where logarithmic-phase cells survive freezing poorly relative to lag- and stationary-phase cells (Hino et al. 1990; Lewis et al. 1993; Park et al. 1997).

An alternative hypothesis considers the role of metabolic waste products, which accumulate during fermentation and are concentrated by freezing (Tanaka and Miyatake 1975; Hsu et al. 1979a). If fermentation wastes are a major cause of freezing injury then the effect of pre-fermentation on the yeast activity of frozen dough might be explained by the level to which wastes accumulate prior to freezing.

The main by-product of fermentative yeast metabolism besides carbon dioxide is ethanol. The inhibitory effects of ethanol on fermentation, viability and growth are predominantly due to alteration of the fluidity and permeability of the plasma membrane, interfering with membrane transport properties (Ingram and Buttke 1984; D'Amore and Stewart 1987).

For example, ethanol inhibits uptake of sugars (Loureiro-Dias and Peinado 1982; Leao and Van Uden 1982; Pascual et al. 1988; Mauricio and Salmon 1992; Salmon et al.

1993), disrupts trans-membrane proton flux (Cartwright et al. 1986; Dombek and Ingram 1987; Pascual et al. 1988) and induces leakage of amino acids (Salgueiro et al. 1988).

Intracellular glycolytic enzymes are vulnerable to the effects of ethanol (Dombek and Ingram 1987; Pascual et al. 1988), and it may have a nonspecific effect on growth via alteration of osmotic pressure (Jones and Greenfield 1986, 1987; Jones 1987b). Other fermentation by-products such as acetaldehyde and medium-chain fatty acids synergistically enhance the harmful effect of ethanol (D'Amore and Stewart 1987; Walker 1998).

Very few studies have examined the relative importance of 'activation' compared with the accumulation of fermentation wastes.

- Tanaka and Miyatake (1975) reported that fermentation wastes accelerated gassing power loss in frozen doughs, especially with pre-fermented yeast cells. Pre-fermented yeast cells in fresh nutrient solution outperformed fresh yeast in pre-fermented solution.

- Tanaka et al. (1976) measured the post-freezing gassing power of yeast suspensions and doughs made with pre-fermented yeast and added ethanol. Gassing power of doughs was inhibited by ethanol but it had a cryoprotective effect in distilled water.

- Hsu et al. (1979a) found that although pre-fermented yeast was more susceptible to freezing damage in broths, adding pre-fermented broth to fresh yeast had a larger impact on gassing power. Volatiles from the broth had a particularly large impact on gassing power, and this was partly reproduced by freezing with ethanol.

In spite of the evidence that volatile fermentation wastes are detrimental to the gassing power of yeast in frozen dough, this research has received little attention.

1.9.4 Freezing injury and gassing power in frozen dough

One unresolved question in the study of frozen dough is how much the loss in gassing power can be attributed to death of yeast cells and how much is due to nonlethal injury. The density of viable yeast cells in dough is commonly assessed by homogenising a piece of dough in diluent, spreading a sample of diluent on an agar plate and counting the number of colonies formed after incubation (Kline and Sugihara 1968; Baguena et

al. 1991; Havet et al. 2000; Stecchini et al. 2002). The usefulness of this method has been questioned for two reasons.

Firstly, separation of yeast cells from the dough matrix is problematic. Godkin and Cathcart (1949) commented that plate counts "tend to be low due to clumping of yeast cells on and within the dough particles."

Secondly, the true parameter measured by plate counting is not 'viability' but 'replicative ability' under the conditions of the agar plate, which are somewhat demanding (Jones 1987a). Meric et al. (1995) remarked that "in a medium such as dough, cellular death is not the sole cause of the loss of gassing power, and nonlethal cryo-damage should also be taken into account." In other words yeast cells may suffer nonlethal damage in frozen dough that precludes them from forming colonies on a plate while retaining the ability to ferment sugars and produce gas in dough (Godkin and Cathcart 1949; Meric et al. 1995). Godkin and Cathcart (1949) concluded that and "the use of standard plate counts for the purpose of determining the survival of vegetative yeast cells subjected to long periods of sub-freezing storage could easily be a faulty and erroneous method to follow."

The difficulty of removing yeast from dough has prompted several workers to develop liquid nutrient broths that simulate fermentation conditions in dough, and from which yeast cells can be removed by centrifuging. The validity of this approach is disputed by some (Dunas 1988).

Atkin et al. (1945) formulated a broth that produced similar gassing power to a flour-water slurry, and their formulation has been used by a number of workers (Koch et al. 1954; Tanaka and Miyatake 1975; Tanaka et al. 1976; Hino et al. 1990). Ling and Hoseney (1977) optimised the earlier formulation and reported that vitamins had little or no effect on fermentation rate, having been supplied in yeast manufacture.

Hino et al. (1990) measured survival of 3 commercial yeast strains pre-fermented for up to 120 minutes in the medium of Atkin et al. (1945) and frozen in water for 7 days. Unfermented cells retained >80% viability and fermentation of 60 or 120 minutes reduced survival to 20-80% and 0-75% respectively.

1.10. Summary and conclusions

Frozen dough presents an economically attractive option for producing fresh-baked bread, but proofing power declines during frozen storage, limiting shelf life. The loss of proofing power is partly attributable to rheological changes that affect gas-holding properties of dough, but the primary concern is to maintain adequate gassing power, i.e. yeast activity.

The decline in gassing power during frozen storage is accelerated by fermentation prior to freezing (pre-fermentation) and shelf life dramatically shortened by pre-fermentation longer than 60 minutes. Guidelines for frozen dough manufacture emphasise the need to minimise pre-fermentation by rapidly processing dough, mixing at low temperature or delaying yeast incorporation. However the mechanisms by which pre-fermentation shortens shelf life are not well understood.

Two main hypotheses have been put forward to explain the effect of pre-fermentation on frozen dough shelf life. The first proposes that the ability of yeast cells to survive freezing associated is with the presence of sufficient trehalose at the time of freezing to mitigate freezing stresses. Trehalose is rapidly broken down during pre-fermentation, and its disappearance corresponds with a loss of freezing resistance in some strains. However trehalose retention often does not correlate with enhanced frozen dough performance, and although trehalose may be a significant factor in the effect of pre-fermentation on frozen dough stability, it is not the only one.

The second hypothesis proposes that volatile waste metabolites accumulate around yeast cells during pre-fermentation and are concentrated to damaging levels during freezing. Few experimental studies have examined this hypothesis; the extent of metabolite accumulation, the specific metabolites involved and their effects on yeast cells are largely unknown.

Developments in the bakery industry have historically been driven by technology rather than science, and this is reflected in the nature of published frozen dough research. It is often empirical in that response variables are predominantly measures of quality such as ‘loaf volume,’ or ‘proof time’ that are important to consumer acceptance of a product but reveal little about the underlying phenomena influencing product characteristics.

This appears to be changing, with improved methodologies and a recognition of the limitations of the technological approach. However economic pressures are also

driving a focus on short-term applied outcomes and reluctance to publish findings that might constitute patentable intellectual property.

The activities of yeast in fermenting dough are not well characterised, and understanding of metabolite fluxes and growth kinetics lags behind knowledge of micro-organisms in other fermented foods. This is partly due to the dense visco-elastic matrix of dough in which yeast cells are dispersed.

2. RESEARCH OBJECTIVES

This work aimed to better characterise the effect of yeast activity on the amount of yeast substrates and metabolic byproducts in dough during fermentation before and after freezing . This was to be done with experimental measurement of yeast metabolites and mathematical modelling of the bulk dough and immediate environs of a yeast cell in dough.

The objectives were:

- Determine the nature and origin of yeast substrates in dough. Measure their consumption during pre-fermentation and after freezing, frozen storage and thawing. Relate substrate consumption to excretion of ethanol and carbon dioxide.
- Mathematically model the uptake of substrate and production of ethanol by yeast in fermenting dough.
- Mathematically model the effect of yeast metabolism on the immediate environment of a yeast cell in fermenting dough. Model the uptake of substrate and production of ethanol as well as the size and duration of solute gradients resulting from yeast metabolism.

3. MATERIALS AND METHODS

3.1. Materials for dough preparation

Chemicals

D-glucose: BDH brand anhydrous general purpose reagent (GPR). BDH Ltd., Poole, England

D-fructose: Sigma brand product number 57-48-7. Sigma Chemical Company Ltd., St Louis, Missouri, USA.

Sucrose: M&B brand Pronalys AR grade. May and Baker Ltd, Dagenheim, England

Maltose: D(+) maltose monohydrate. United States Biochemical Corporation, Cleveland, Ohio, USA.

Ethanol:

- for recovery experiments and assay standards: Merck absolute ethanol, 99.8% purity
- for experiments to measure ethanol impact on fermentation: 95% pure general purpose reagent (GPR).

Flour

Champion brand high grade white flour (Goodman Fielder, Auckland, New Zealand) containing 11.5% protein (manufacturer's data) and 12.0% moisture (data in Appendix A2). Purchased from a Palmerston North supermarket in 5kg double-walled paper bags and stored at room temperature.

Yeast

For gassing power experiments 1kg blocks of Pinnacle fresh compressed yeast were purchased from Pak 'n' Save supermarket in Palmerston North, transported to Massey University in a polystyrene bin within an hour of purchase and stored at 5˚C. At the time of use yeast was not more than 20 days beyond the date of manufacture.

For yeast metabolite experiments 1kg blocks of Pinnacle brand fresh compressed yeast were supplied on the day of manufacture by New Zealand Food Industries,

Auckland, New Zealand. Blocks were couriered to Palmerston North in a polystyrene bin with an ice pack and stored at 5˚C. At the time of use yeast was not more than 14 days beyond the date of manufacture.

Water

Reverse-osmosis filtered water, Massey University laboratory supply.

Salt

Cerebos brand iodised table salt, 300 g plastic container purchased from a Palmerston North supermarket.

Oil

Basics brand canola oil, 2 litre plastic bottle purchased from a Palmerston North supermarket.

3.2. Materials for enzymatic assays

Boehringer Mannheim / R-Biopharm Enzymatic BioAnalysis kits (Roche Diagnostics, Mannheim, Germany):

- Maltose/Sucrose/D-Glucose (order number 11 113 950 035)
- Alcohol universal kit (order number 1776312)

Glucose hexokinase reagents: Gluco-quant, order number 11 447 513 216 (Roche Diagnostics, Mannheim, Germany).

Calibrator for automated systems (C.f.a.s) order number 10 759 350 (Roche Diagnostics, Mannheim, Germany)

Low control serum: Precinorm U, order number 10 171 743 122, (Roche Diagnostics, Mannheim, Germany).

High control serum: Precipath U, order number 10 171 778 122, (Roche Diagnostics, Mannheim, Germany).

Fructosidase: beta-fructosidase from yeast, order number 104 914 (Roche Diagnostics, Mannheim, Germany).

Fructosidase buffer: 50 mmol.L^{-1} citrate buffer, pH 4.6. Massey University laboratory supply.

Ethanol controls – any of the following solutions diluted with water to give 1.5 mmol.L^{-1}:

- Ammonia/Ethanol/CO_2 calibrator, order number 20 751 995 190 (Roche Diagnostics, Mannheim, Germany).
- Ethanol assay control from Boehringer Mannheim / R-Biopharm Enzymatic BioAnalysis kits, order number 10 176 290 035 (Roche Diagnostics, Mannheim, Germany).
- control solution freshly made with Merck absolute ethanol

3.3. Equipment for dough preparation and analysis

3.3.1 Breadmaker

Breville Bread Master Big Loaf breadmaker (Breville Holdings Pty Ltd.).

Figure 3.1. Breville breadmaker.

3.3.2 Freezing, frozen storage and thawing

Neslab RTE 140 Refrigerated Bath/circulator with digital controller (Thermo Electron Corporation, New Hampshire USA).

Nylon hoses: external diameter 10 mm, internal diameter 8 mm (Plumbing World, Palmerston North).

Foam insulation for hoses.

Steel hose clamps (Plumbing World, Palmerston North).

Valves: Topic brand globe valves with ¼ inch male fitting and hosebarb (Plumbing World, Palmerston North).

Stainless steel tube, external diameter 9 mm internal diameter 8 mm, custom-made.

Coolant: 95% pure ethanol or 50:50 mixture of water and Preston antifreeze/coolant (Preston Products Corporation, Danbury CT, USA).

Water baths:

- freezing – stainless steel water bath 260 mm x 310 mm x 150 mm insulated with 25 mm polystyrene on sides and bottom.
- thawing – stainless steel water bath 520 mm x 310 mm x 150 mm

Frozen storage: Frigidaire food freezer (domestic chest freezer), General Motors.

Stirrer motor: Heidolph, model unknown (Watson Victor, New Zealand).

Stirrer: stainless steel shaft with 35 mm diameter two-bladed propeller, custom-made at Massey University.

Plastic bags: Glad brand Snap lock.

Lead fishing weights, 8 ounce.

Retort stand and clamps

Grant Squirrel 2020 datalogger (Grant Instruments, Cambridgeshire, England) and T-type thermocouple wires.

3.3.3 Weighing

Mettler AE 260 Deltarange balance

Mettler BB2400 balance

Mettler Toledo BB3002 balance

3.3.4 Gassing power measurement

Risograph model 2E supplied with stainless steel cans, lids, weights and connecting hoses (R-Design, Pullman, Washington, USA).

Tandy 102 portable computer (Tandy Corporation, Fort Worth, Texas, USA)

Custom-made serial data transfer cable with the following pin configuration:

Tandy 102	PC
25 pin male	9 pin female
2	2
3	3
7	5

Figure 3.2. Risograph and accessories.

3.3.5 Dough extraction and analysis

Waring Commercial Blendor with 600 ml stainless steel cup and plastic lid (Watson Victor, New Zealand).

Aluminium moisture dish, 65 mm diameter.

Polypropylene centrifuge tubes, 50ml (Biolab Scientific, Auckland, New Zealand).

Homogeniser: Heidolph Diax 600 with 18mm diameter blade type 18G (Heidolph, Germany).

Water heater: Techne model FTE 10AE

Syringes: single use plastic, 5ml (Terumo).

Disposable filters: Minisart, pore size 0.8µm, 26 mm diameter, order number 16592 (Sartorius AG, Goettingen, Germany).

Plastic tubes: 1.5ml (Eppendorf Safe-lock, Eppendorf AG, Germany)

Dry ice pellets (solid carbon dioxide) supplied by BOC gases, Palmerston North.

Freeze-ground dough storage: -86C Freezer, Forma Scientific.

Centrifuge: Centra MP4R, IEC. Rotor number 851.

Cobas Fara II robotic transfer analyser with sample tubes, reagent cups and cuvette rotors (Roche Diagnostics, Mannheim, Germany).

3.4. Method for dough preparation

Doughs were made in 1 or 1.5kg batches according to the formulation in Table 3.1. For some experiments doughs were made without yeast, with different sugars and/or with added ethanol. These doughs contained the same amount of flour, water, oil and salt (Table 3.1), and differences in the total mass were accounted for in calculations.

Table 3.1. Basic formulation for dough manufacture.

Ingredient	*Weight (g)*	
	1kg batch	*1.5kg batch*
flour	600	900
water	330	495
yeast	20	30
canola oil	20	30
sucrose	20	30
iodised salt	10	15

Ingredients were combined in the pan of a Breville Breadmaster Big Loaf breadmaker (Breville Holdings Pty Ltd.) (Figure 3.1). Water was added to the pan first then oil, flour, salt, sugar and yeast. Compressed yeast was broken up by hand as it was added on top of other ingredients. In experiments testing the recovery of sugars from dough, sugar was completely dissolved in the water before other ingredients were added to the pan.

Doughs were mixed for 18 minutes using automatic setting 1 labeled 'Basic' and 'Medium' crust colour setting. Dough temperatures were typically 26 to 35˚C at the end of mixing.

Batches of dough were divided by hand into 50 g subsamples (hereafter referred to as 'doughs') and placed in snap lock plastic bags. Doughs were shaped by hand into slabs approximately 1cm thick and the snap lock was closed.

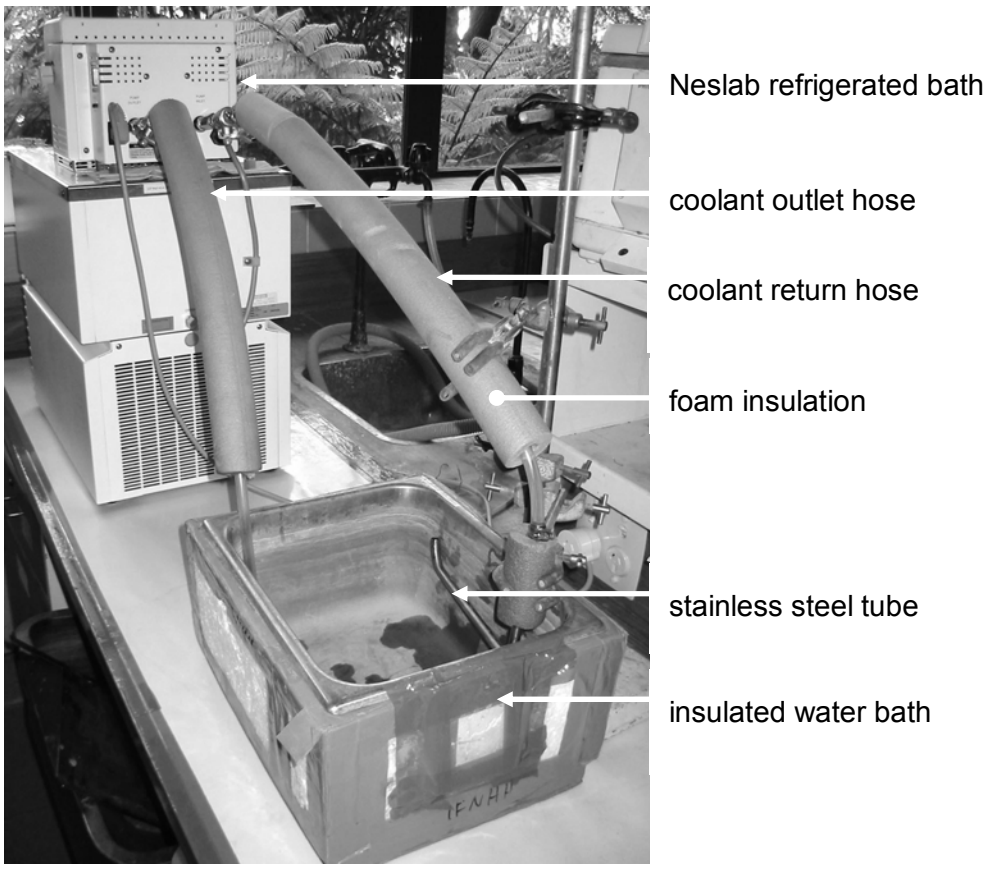
Doughs to be pre-fermented were sealed in plastic bags, shaped into slabs and laid flat on wire racks in a temperature-controlled room at 30˚C.

3.5. Methods for freezing and frozen storage

Freezing was performed in the Neslab RTE 140 refrigerated bath. For small experiments, the bath work area was sufficiently large to fit all doughs, but for larger experiments coolant was recirculated to an external bath insulated with polystyrene, and doughs were frozen both in the bath work area and the external bath (Figure 3.3).

Subsamples of 50g dough were packaged in a double layer of plastic bags with a lead weight in the outer bag. Bags were submerged in the refrigerated bath with the coolant level above the level of the dough but below the top of the plastic bags.

Figure 3.3. Freezing bath coolant recirculation equipment.



Doughs were frozen at -20˚C in the refrigerated bath for 60 minutes then the outer plastic bag was discarded and samples were transferred to the Frigidaire freezer at -18˚C.

After the required period of storage doughs were placed inside a weighted plastic bag and thawed for 60 minutes in a water bath containing ice-water slush stirred with a motorised stirrer. Thawed doughs were transferred to a temperature-controlled room at 30˚C.

Temperature profiles of 50g doughs during freezing at -20˚C and warming from 0˚C to 30˚C in the temperature-controlled room were recorded with T-type thermocouples. These were inserted at the approximate centre of the sample and connected to the Squirrel data logger, which recorded temperatures once every minute. Thermocouples were calibrated in partly frozen reverse osmosis water. Data are shown in Appendix A4.

3.6. Method for measuring gassing power

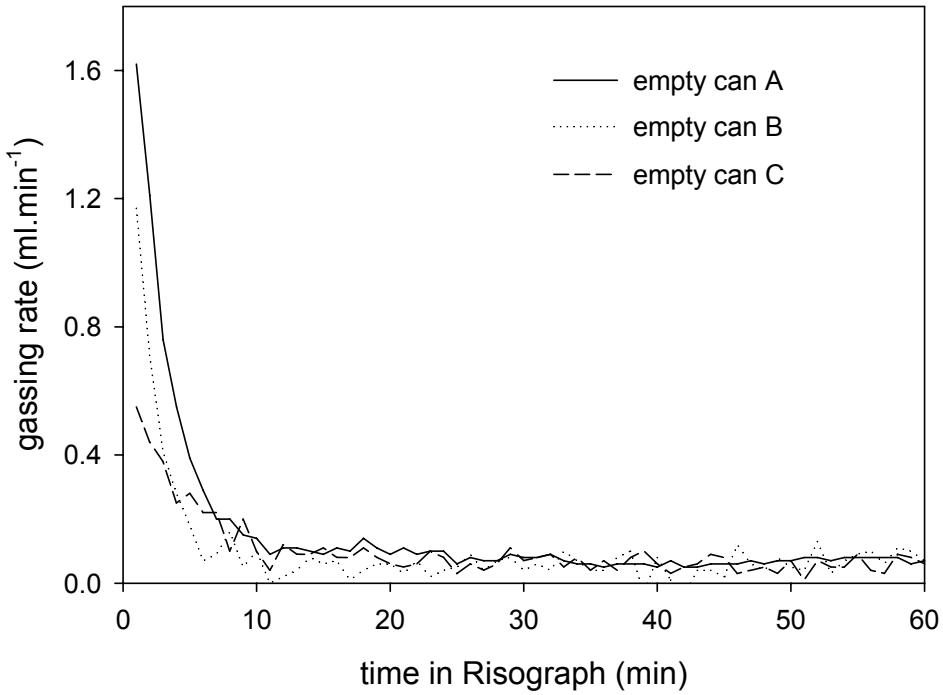
The Risograph was calibrated and leak-tested according to the manufacturer's protocol.

Doughs were weighed to within 3 g of 50 g, sealed in stainless steel cans, placed in a water bath at 30˚C with a weight on top of each can and connected to the Risograph chassis with plastic hoses. Gas volumes were recorded at one-minute intervals.

Cumulative gas volume or gassing rate data were transferred from the Tandy 102 to a personal computer running Microsoft Windows using programs supplied by the Risograph manufacturer. The data transfer was through a custom-made cable connected to the RS-232 serial port on the Tandy and a 9-pin serial port on the personal computer.

The Risograph measures the volume of gas produced by each dough sample in a set time interval. Thermal expansion of air in cans equilibrating to the water bath temperature can cause recorded gas production to be larger than its true value. This is illustrated by the gassing rate recorded on empty cans (Figure 3.4).

Figure 3.4. Apparent gas production from empty cans recorded by the Risograph.



The most rapid expansion was during the first 10 minutes. Gas production data was corrected for thermal expansion by discarding the first 10 minutes of data, as recommended by the Risograph manufacturer.

Gassing curves were summarised with the parameter 'mean volume' (Meric et al. 1995), which is the area under the cumulative gas volume curve divided by the time over which the curve was recorded. For data sets of one reading per minute, the area under the curve is well-approximated by the sum of cumulative volume readings. Mean volume favourably weights initial rapid gassing power due to the cumulative calculation (Meric et al. 1995).

A statistical comparison of preliminary data showed that mean volume discriminated between treatments better than average gassing rate, maximum gassing rates or total gas volume.

3.7. Method for measuring sugars and ethanol in dough

3.7.1 Dough extraction

Dough was torn into small pieces by hand and dropped into liquid nitrogen in a pre-cooled aluminium moisture dish. Once the liquid nitrogen had stopped boiling vigorously the dough and remaining liquid nitrogen were tipped into a pre-cooled Waring blender jar. A lid was place on top of the cup and the blender started on speed two. The lid was held on with sufficient force to prevent dough being thrown out of the jar but not enough to allow a pressure build-up from the boiling liquid nitrogen.

Quench-frozen doughs were shattered in the Waring blender on speed two for 10 seconds then speed 1 for 10 seconds, producing a fine powder. Dough powder was stored in plastic bags on dry ice while other samples were being freeze-shattered (several hours) and transferred to a freezer at -80˚C for long-term storage.

Centrifuge tubes were filled with 25 ml water and 2.5 g dough powder accurately weighed into each tube using a metal spoon pre-cooled in dry ice. The homogeniser shaft was submerged to the bottom of the tube and the slurry homogenised at 13500 rpm for 30 seconds.

Approximately 4 ml of the homogenised slurry was sucked into a 5 ml syringe, a Minisart filter attached to the syringe tip and 1 ml filtrate expelled through the filter into 1.5 ml plastic tubes.

Unheated extracts were transferred to a freezer at -20˚C shortly after extraction.

Extracts to be heated were pushed through a sheet of polystyrene floating on top of a water bath at 85˚C. After one hour heated extracts were cooled on ice for 10 minutes then centrifuged at 13,200 rpm for 5 minutes. Supernatants were pipetted to fresh 1.5 ml tubes and transferred to a freezer at -20˚C.

3.7.2 Enzymatic assays

Assays were performed on a robotic COBAS Fara II transfer analyser using analytical protocols developed at the Massey University Nutrition Laboratory. Preparation of assay reagents and details of analyser protocols are shown below.

Enzyme assays were calibrated on the day they were carried out using Roche 'Calibrator for automated systems', low control Precinorm U and high control Precipath U. Calibrator and controls were frozen between uses, according to the manufacturer's guidelines.

200-400 µl dough extract was pipetted from 1.5 ml tubes into COBAS analyser sample cups in racks. A rack of samples was placed on the analyser and the assay program started.

Assay results were converted from mmol.L^{-1} extract to mmol.(100g dough)$^{-1}$:

$$\frac{mmol\ analyte}{L\ extract} \cdot \frac{0.025L\ water}{x\ grams\ dough} \cdot 100 \qquad 3.1$$

Glucose

Reagents:

Glucose reagent – Gluco-quant reagents 1 and 2 were mixed in the proportion 5:1. Reagent was made freshly each day or refrigerated at 4˚C for up to 3 days, according to the manufacturer's guidelines.

COBAS protocol:

1. Pipette 4 µl sample into cuvette
2. Dilute with 30 µl H_2O
3. Pipette 200 µl glucose reagent into cuvette
4. Spin rotor to mix
5. Incubate at 37˚C measuring absorbance at 340 nm at 10 second intervals for 3 minutes.

Fructose

Reagents:

Glucose reagent

Fructose reagent – PGI suspension from BioAnalysis kit diluted 1:1 with water. Diluted on the day of use.

COBAS protocol:

1. Pipette 3µl sample into cuvette
2. Dilute with 50 µl water
3. Pipette 200 µl glucose reagent into cuvette
4. Spin rotor to mix
5. Incubate at 37˚C for 2.5 minutes
6. Pipette 5 µl fructose reagent into cuvette
7. Dilute with 15 µl water
8. Spin rotor to mix
9. Incubate at 37˚C measuring absorbance at 340 nm at 60 second intervals for 5 minutes.

Sucrose

Reagents:

Glucose reagent

Sucrose reagent – beta-fructosidase made up on the day of use at 5 $mg.ml^{-1}$ in 50 $mmol.L^{-1}$ citrate buffer, pH 4.6.

COBAS protocol:

1. Pipette 2µl sample into cuvette
2. Dilute with 10 µl water
3. Pipette 10 µl sucrose reagent into cuvette
4. Dilute with 10 µl water
5. Spin rotor to mix.
6. Incubate at 37˚C for 6 minutes
7. Dilute with 50 µl water
8. Pipette 250 µl glucose reagent into cuvette
9. Spin rotor to mix
10. Incubate at 37˚C, measuring absorbance at 340 nm at 45 second intervals for 3.75 minutes

Maltose

Reagents:

Glucose reagent

Maltose reagent – α-glucosidase from BioAnalysis kit made up in water at 30 $mg.ml^{-1}$ on the day of use.

Standard solution of 5 $mmol.L^{-1}$ maltose in water made freshly each day.

COBAS protocol:

1. Pipette 2 µl sample into cuvette
2. Dilute with 15 µl water
3. Pipette 13.5 µl maltose reagent into cuvette
4. Dilute with 13.5 µl water
5. Spin rotor to mix
6. Incubate at 37˚C for 6 minutes
7. Dilute with 25 µl water
8. Pipette 300 µl glucose reagent into cuvette
9. Spin rotor to mix
10. Incubate at 37˚C measuring absorbance at 340 nm at 60 second intervals for 6 minutes.

Ethanol

Ethanol reagent: Alcohol kit reagents 1 and 2 mixed in 1:1 ratio. Made up on the day of use or refrigerated for up to five days.

COBAS protocol:

1. Pipette 60 µl sample into cuvette
2. Dilute with 10 µl water
3. Pipette 150 µl ethanol reagent into cuvette
4. Incubate at 37˚C measuring absorbance at 340 nm at 25 second intervals for 5.5 minutes.

3.8. Moisture determination

Samples of flour (between 2 and 3 grams) or yeast (between 3 and 6 grams) were accurately weighed into dry aluminium moisture dishes (diameter 65mm) and dried for 2-3 days at 108˚C in an oven (Watvic oven, Watson Victor, New Zealand). Dishes were removed from the oven and allowed to cool in desiccators then weighed.

3.9. Data analysis and presentation

Yeast metabolite calculations and Risograph data analysis were performed in Microsoft® Office Excel 2003 (Microsoft Corporation).

Analysis of variance (ANOVA), linear regression analysis and paired comparisons were performed in Minitab® Release 14 (Minitab, Inc.).

Mathematical modelling used Matlab version 6.5 release 13 (The MathWorks, Inc.).

Data were presented graphically using Sigmaplot 2004 for Windows version 9.01 (Systat Software, Inc.).

Means and standard deviations were calculated in Excel, Minitab and Sigmaplot using equations 3.2 and 3.3 respectively. Standard errors were calculated with equation 3.4.

$$\bar{y} = \frac{\sum y}{n} \qquad 3.2$$

$$s = \sqrt{\frac{\sum (y - \bar{y})^2}{n - 1}} \qquad 3.3$$

$$SE = \frac{s}{\sqrt{n}} \qquad 3.4$$

s sample standard deviation

n sample size

y individual response value

$\bar{y}$ sample mean

4. WATER RELATIONS IN THE PRESENT DOUGH SYSTEM

Information in the literature showed that water molecules in dough interact with polymers in such a way that their physical properties are very different from water molecules in pure liquid water. There is good agreement between authors using different methods that the interacting or 'non-solvent' water in flour-water doughs constitutes approximately 0.3 grams of water per gram of flour dry matter (section 1.7).

The remainder of dough water has mobility typical of pure water and forms an aqueous phase surrounding the gluten network. The division between solvent and non-solvent water is not sharp because water molecules can occupy any point along a spectrum of mobility states (Ruan et al. 1999; Kou et al. 2002; Esselink et al. 2003). However a single figure of solvent water content was needed for modelling purposes.

It was assumed that literature data would be applicable to doughs made here, and the amount of solvent water was not verified. The total amount of water in dough was calculated from the formulation and the moisture contents of flour and compressed yeast (Table 4.1).

Table 4.1. Water supplied by dough ingredients.

source	*% w/w in dough*	*% moisture*	*grams water per 100 grams dough*
flour	60	12.0	7.20
water	33	100	33.00
yeast	2	67.3	1.35
total moisture[A]			41.55
solids			58.45
			100.00

A. excluding minor amounts from oil, sugar and salt

Added salt and sugar enhance water sorption in dough, either by directly interacting with water molecules or by altering protein structure and the orientation of hydrophilic residues (Laaksonen and Labuza 2001; Laaksonen and Roos 2003). There was insufficient information in the literature to judge how much extra water would be

adsorbed by added solutes, and this was not accounted for in calculation of solvent water.

If flour dry matter (total mass minus moisture, 58.45 g.(100g dough)$^{-1}$) took up 0.3 grams of water per gram the remaining solvent water would be 41.55 – 0.3 x 58.45 = 24.0 g.(100g dough)$^{-1}$. This was assumed to have the density of 1.0 g.ml^{-1}, and gravimetric solute concentrations in dough were converted to volumetric concentrations on the basis of 24.0 ml.(100g dough)$^{-1}$ solvent water:

$$\frac{mmol}{100g\ dough} \times \frac{100g\ dough}{0.0240L} = \frac{mmol}{L} \qquad 4.1$$

The rheological properties of dough change during fermentation (Lee et al. 2004), indicating significant structural changes in the dough. However no information was found on changes in water mobility or osmotic properties of dough during fermentation. The solvent water content calculated above was therefore assumed to apply throughout fermentation. Doughs in this work contained 1% salt and up to 2% sucrose, 2% fructose or 4% glucose. According to the above calculation there was sufficient solvent water to completely dissolve salt and sugars.

5. FACTORS AFFECTING PRE-FREEZING FERMENTATION RATE

5.1. Introduction

The amount and type of sugar in dough formulation is known to affect the rate at which yeast ferments (Schultz 1965; Suomalainen et al. 1972; Saalfeld and Freund 1998). The rate of fermentation in the dough system used here was measured with a range of sugar types and levels to find an optimum formulation in which sugar was not limiting or inhibiting fermentation.

High levels of ethanol are injurious to yeast cells due to their disruptive effect on membrane lipids (section 1.9.3). This section reports the effect of exogenous ethanol on fermentation rate in the present dough system. These experiments were done to give an indication of the level at which endogenous ethanol accumulated during fermentation would begin to inhibit yeast cell metabolism.

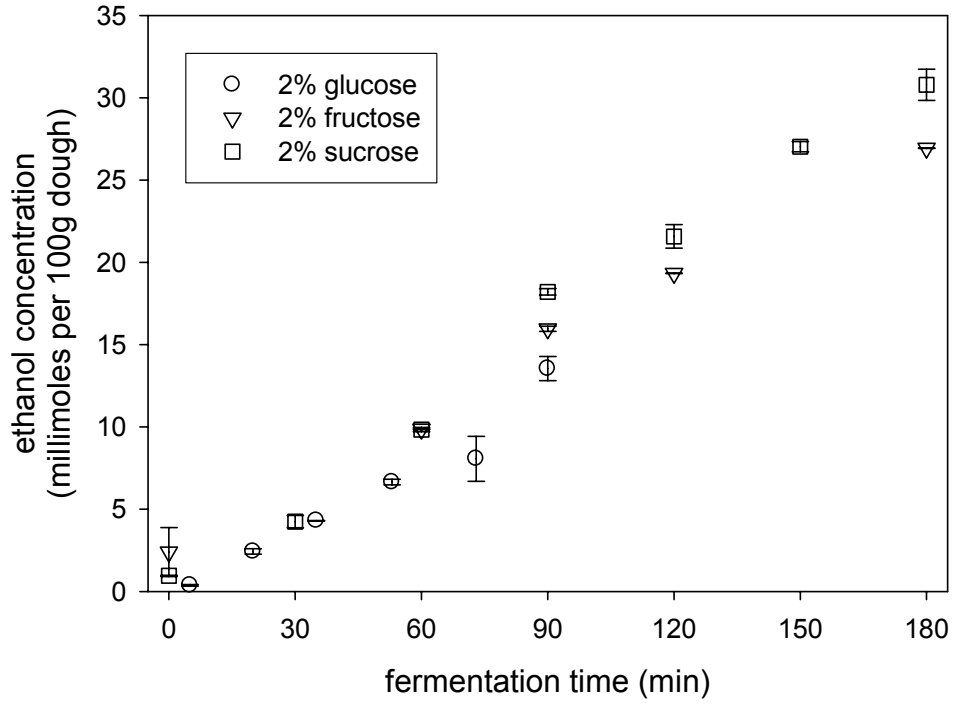
These experiments were designed to optimise dough formulation and put endogenous ethanol levels in context, and as such they did not have a high degree of replication or statistical rigour.

5.2. Impact of sugar source on fermentation rate

Three yeasted doughs were made with either 2% glucose, fructose or sucrose as the added sugar source. Gas production over two hours was measured in the Risograph and each dough was assayed for ethanol during fermentation at 30˚C.

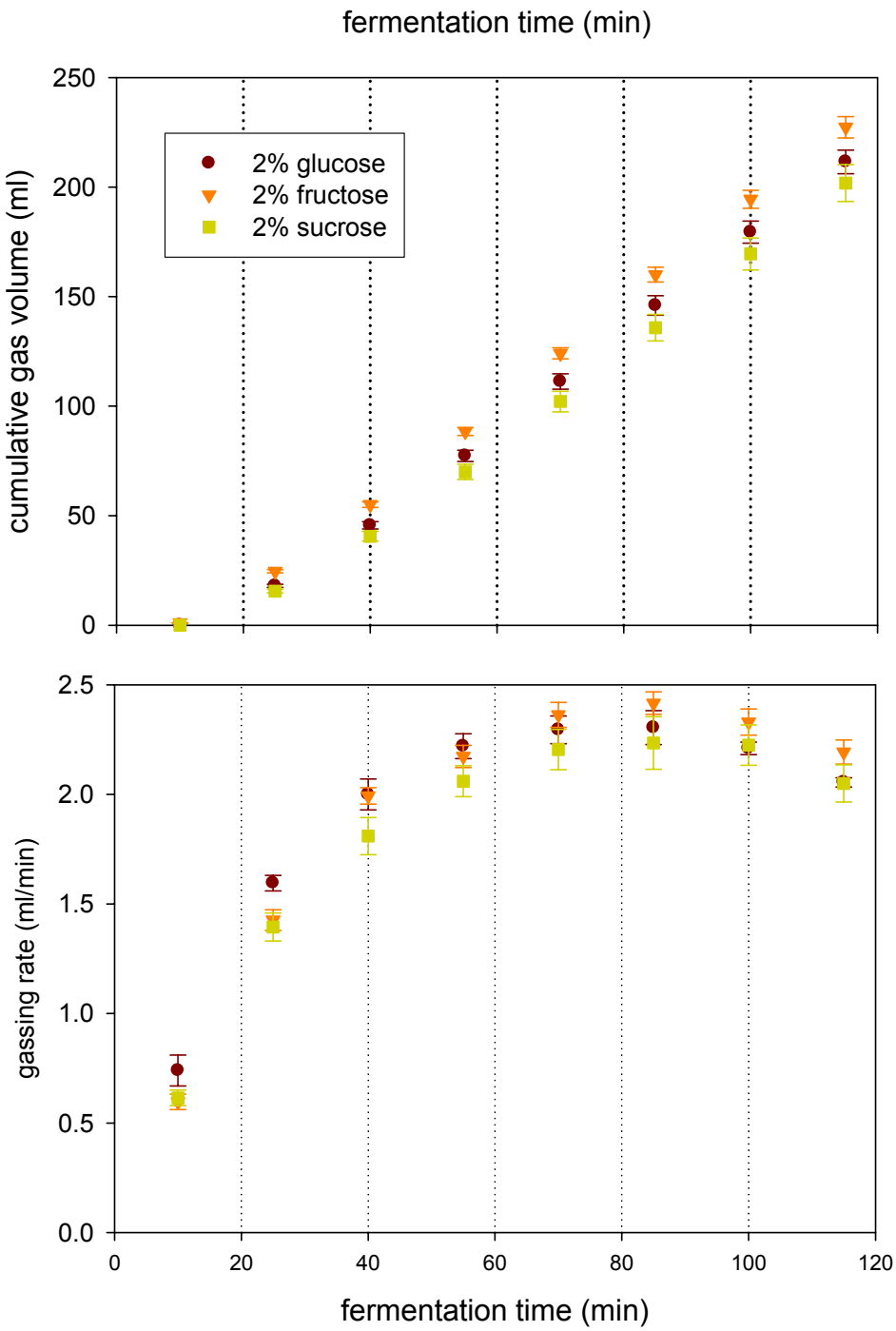
Ethanol accumulated at a constant rate in all doughs, and differences among doughs appeared to be minor (Figure 5.1). Gassing power differences were also small (Figure 5.2), in agreement with other findings (Schultz 1965), which confirmed that yeast cells fermented glucose, sucrose and fructose at very similar rates.

Figure 5.1. Effect of sugar source on ethanol production in dough fermenting at 30˚C.



Each data point is the mean of at least two assay results. Vertical bars are standard deviations.

Figure 5.2. Effect of sugar source on gas production by doughs fermenting at 30˚C.



Vertical bars are the standard deviations from two or three 50-gram subsamples. Data collected during the first ten minutes were discarded to allow for thermal expansion of air in Risograph cans.

5.3. Impact of glucose level on fermentation rates

Doughs were made with 0.5, 1.0, 2.0 or 4.0% added glucose and subjected to gassing power analysis. Ethanol was measured in 0.5% and 2.0% glucose doughs, and increased linearly in both (Figure 5.4).

The impact of sugar level on gas production was shown best by gassing rates data (Figure 5.3). The general pattern of gas production was similar – a rapid initial increase in gassing rate, a plateau then a decline. The initial increase was similar in doughs with 2% or less glucose, but with 4% it was somewhat slower.

The gassing rate of the 0.5% glucose dough reached a lower maximum than other doughs and it declined quickly thereafter. Doughs with 2.0% or 4.0% glucose took 80-90 minutes to reach maximal gassing rate while other doughs peaked earlier. At the lower sugar levels gassing rate appeared to reach a second plateau at around 1.5 $ml.min^{-1}$ after the post-maximal decline.

While there were significant variations in gassing rate over two hours and gas production was optimal with 1.0-2.0% glucose, cumulative volume differences were relatively small.

The rates of ethanol accumulation and glucose consumption in doughs with 0.5% or 2.0% added glucose were approximately constant over the first 60 minutes of fermentation (Figure 5.4). Glucose was consumed at equal rates in the two doughs but ethanol was produced faster in the dough with 0.5% added glucose (Table 5.1).

Table 5.1. Effect of glucose level on glucose consumption and ethanol production rates calculated with linear regression.

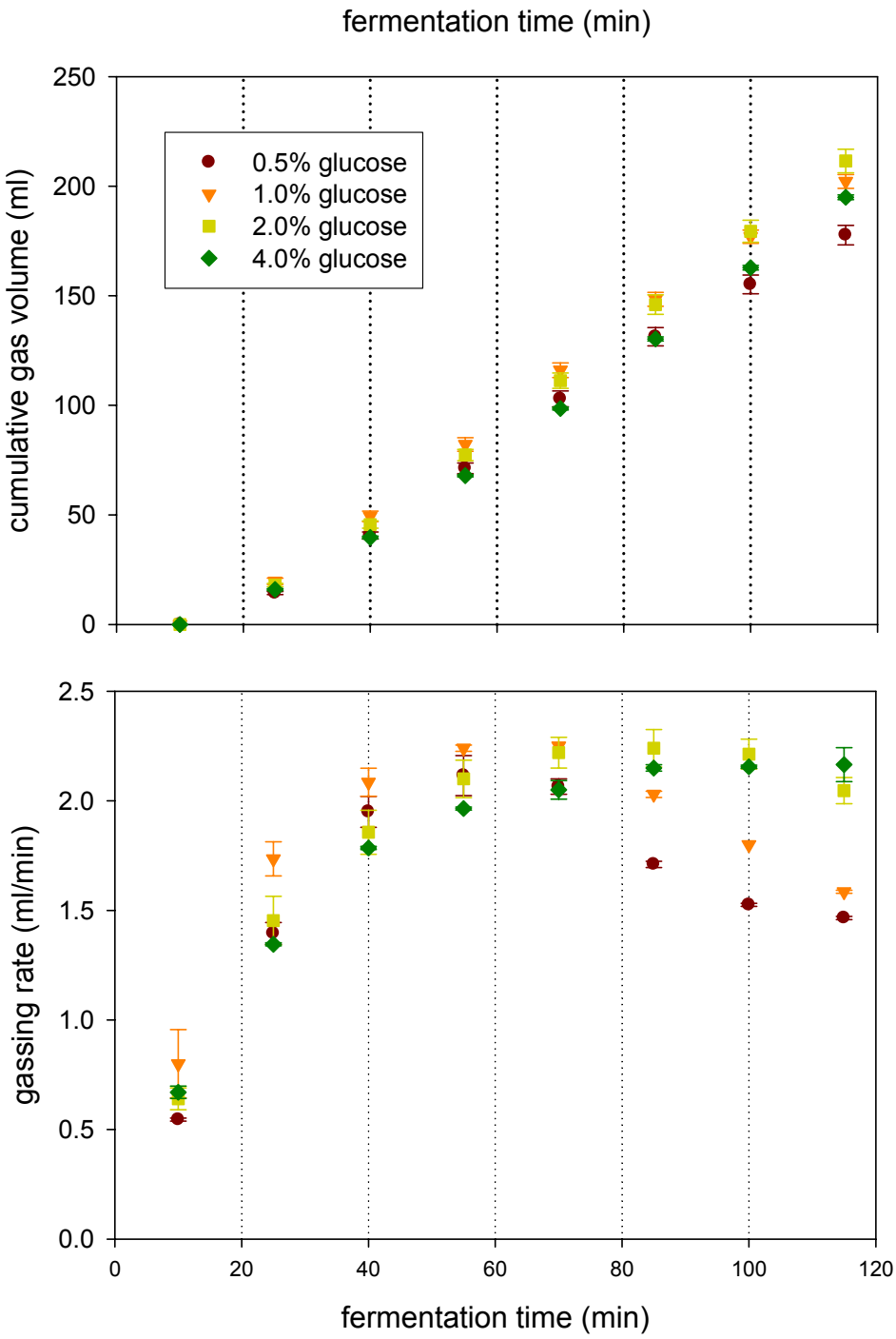
	glucose consumption					*ethanol production*				
added glucose	*n*[A]	*r-sq*	*slope*	*SE*[B]		*n*	*r-sq*	*slope*	*SE*	
2.0%	24	98.8	-0.0710	0.0017	a[C]	11	99.9%	0.129	0.001	b
0.5%	10	78.5	-0.0576	0.0106	a	8	99.9%	0.164	0.004	a

A. number of data points

B. standard error of the slope

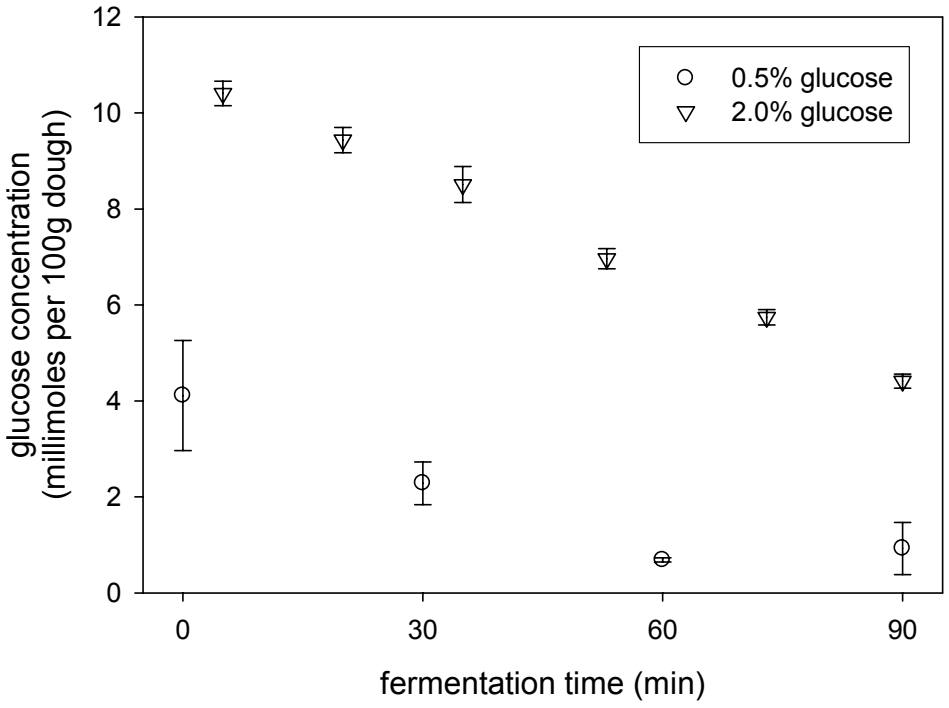
C. rows with the same letter were not significantly different at 95% confidence

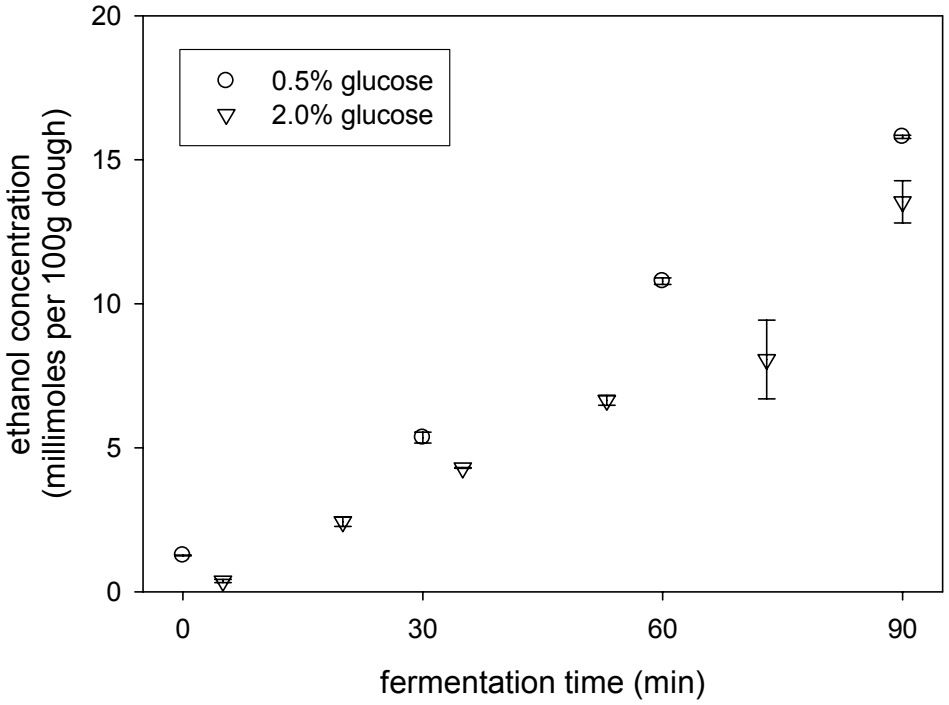
Figure 5.3. Effect of glucose level on gas production in doughs fermenting at 30˚C.



Vertical bars are standard deviation from duplicate 50g dough samples. Data collected during the first ten minutes were discarded to allow for thermal expansion of air in the Risograph can.

Figure 5.4. Effect of added glucose level on glucose consumption (top) and ethanol production (bottom) in doughs fermenting at 30˚C.



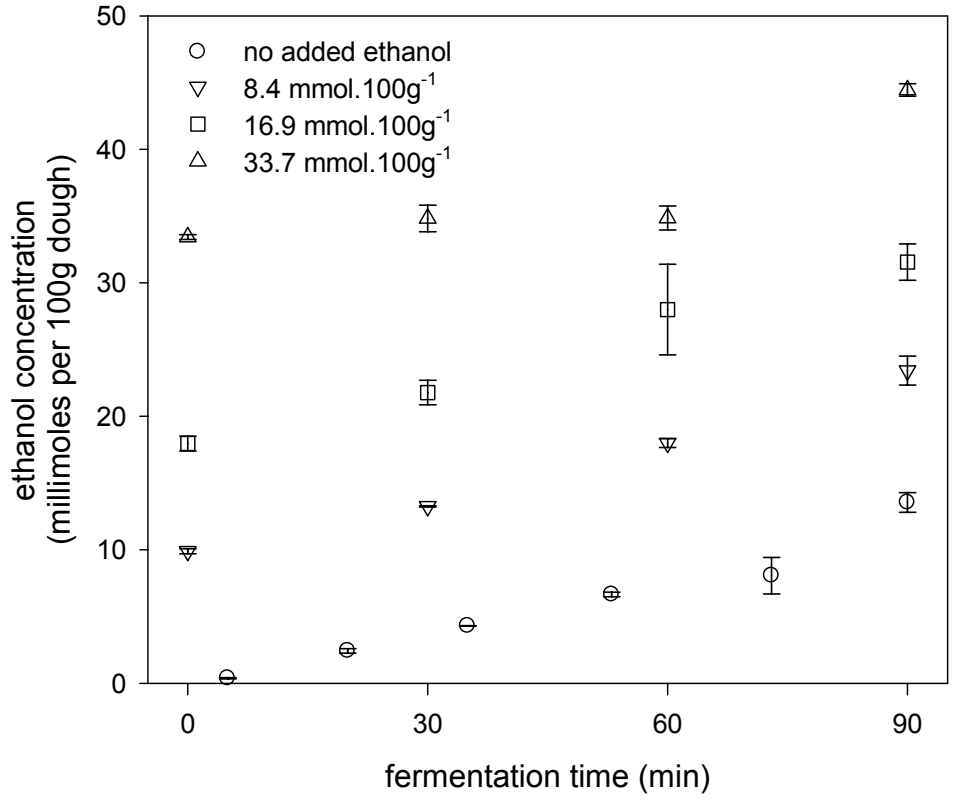


Vertical bars are the standard deviations of duplicate assay results. Duplicates were not averaged for regression analyses.

5.4. Impact of added ethanol on fermentation rates

Doughs were made with 2% glucose and 95% ethanol was added at 5, 10 or 20 ml.kg^{-1}, which corresponded to 8.4, 16.9 or 33.7 mmol.(100g dough)$^{-1}$. Ethanol was mixed into the water in the breadmaker pan before dry ingredients were added and the dough mixing process began.

Figure 5.5. Effect of added ethanol on ethanol production in doughs fermenting at 30˚C.



Vertical bars are standard deviations from at least two assay results. Doughs were made with 2% added glucose.

Doughs contained slightly more than the added amount of ethanol (Figure 5.5), probably because some fermentation had occurred during mixing. With 33.7 mmol.(100g dough)$^{-1}$ added ethanol, there was no increase in ethanol over the first hour, but in other doughs it accumulated at a constant rate over 90 minutes. Data were analysed with linear regression analysis (Table 5.2).

In dough with 16.9 mmol.(100g dough)$^{-1}$ added ethanol, the rate of ethanol production was not significantly different from the dough without added ethanol. Ethanol was produced faster with 8.4 mmol.(100g dough)$^{-1}$ added ethanol than without added ethanol (Table 5.2). Ethanol accumulation in the dough with 33.7 mmol.(100g dough)$^{-1}$ was not linear during 90 minutes of fermentation, and regression analysis was not applied.

Table 5.2. Effect of added ethanol on ethanol production rates.

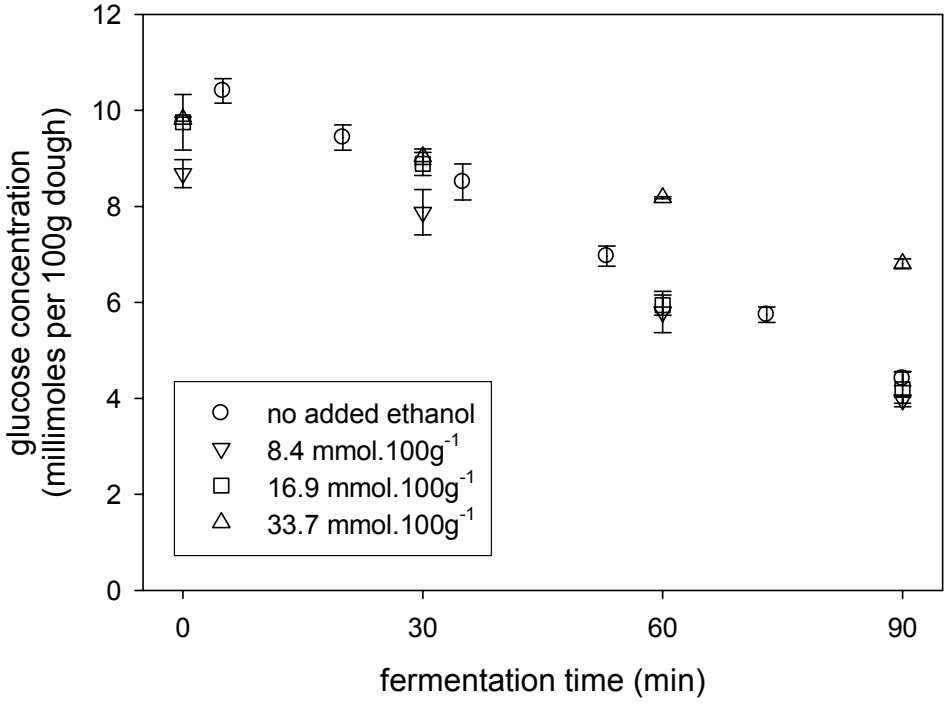
added ethanol	*n*[A]	*r-squared*	*slope*	*SE*[B]	
none	11	99.9	0.129	0.001	b[C]
8.4 mmol.(100g dough)$^{-1}$	16	98.0	0.151	0.006	a
16.9 mmol.(100g dough)$^{-1}$	16	90.1	0.157	0.014	ab

A. number of data points

B. standard error of the slope

C. rows with the same letter were not significantly different at 95% confidence

Figure 5.6. Effect of added ethanol on glucose consumption in doughs fermenting at 30˚C.

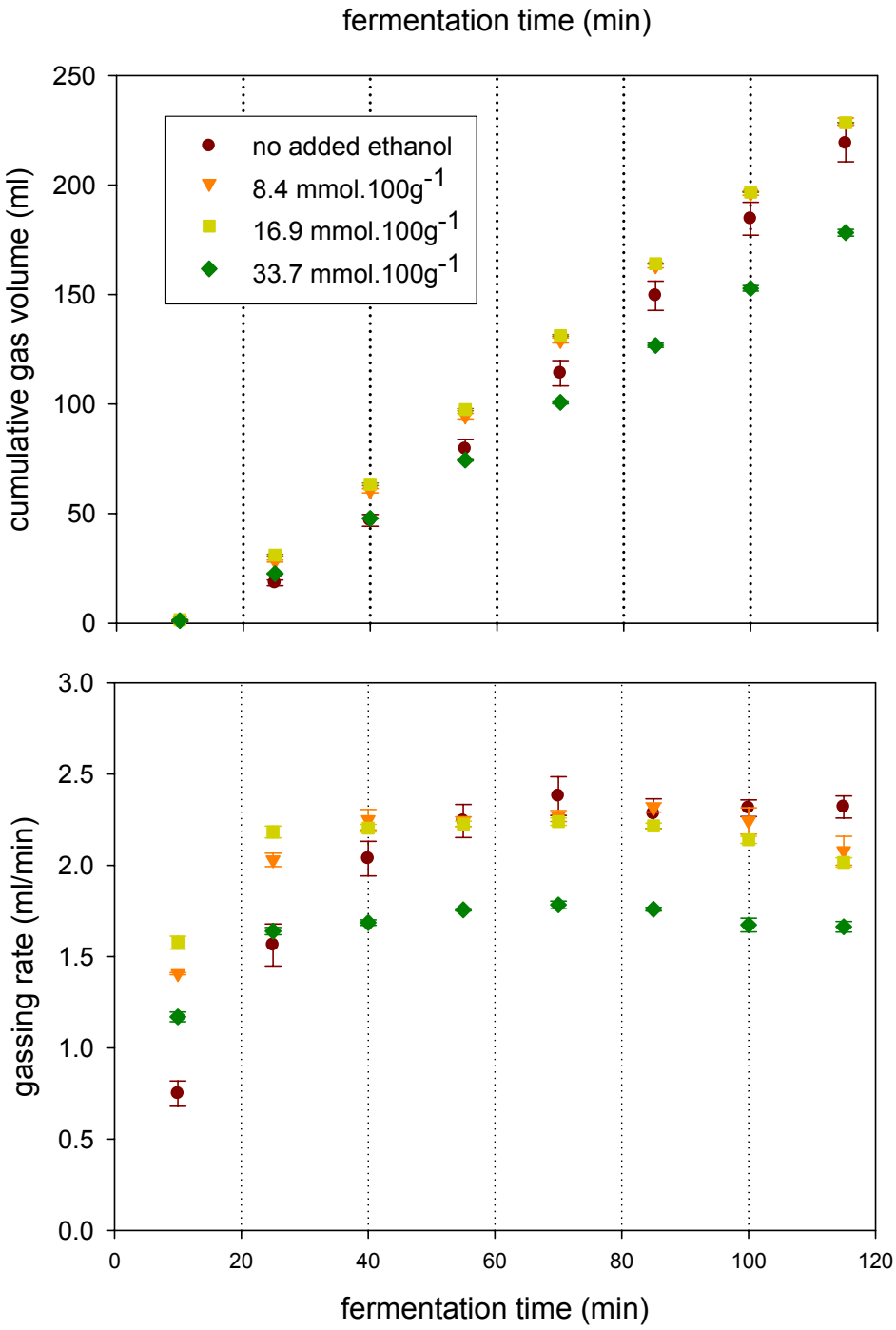


Vertical bars are standard deviations from at least two assay results.

Within experimental error, glucose was consumed at approximately the same rate for all levels of added ethanol except 33.7 mmol.(100g dough)$^{-1}$ (Figure 5.6). With the highest level of added ethanol, glucose declined progressively at a rate slower than in other doughs.

Adding ethanol at 5 or 10 ml.kg^{-1} appeared to stimulate gas production during the first hour of fermentation (Figure 5.7). Maximal gassing rate was slightly lower in these doughs, and markedly lower in the dough with 20 ml.kg^{-1} added ethanol. Gassing rate data showed a superimposed sinusoidal fluctuation of unknown origin.

Figure 5.7. Effect of added ethanol on gas production.



Top: cumulative gas volume, bottom: gassing rate. Doughs were made with 2% added glucose. Each curve is the mean from three 50g dough samples and vertical bars are standard deviations. Data collected during the first ten minutes were discarded to allow for thermal expansion of air in Risograph cans.

5.5. Summary and conclusions

Sucrose is rapidly hydrolysed in yeasted doughs, producing an equimolar mixture of glucose and fructose (Koch et al. 1954; Varo et al. 1979; Rogers and Langemeier 1995). Maximal uptake rates of either sugar alone are very similar in broth culture (Serrano and De la Fuente 1974), brewing fermentations (D'Amore et al. 1989) and bakery fermentation (Varo et al. 1979). This work showed that these findings were applicable to the current dough system and that the equivalence in uptake rates was reflected in ethanol and carbon dioxide production.

Some studies on liquid fermentation systems (Orlowski and Barford 1987; Wang et al. 2004) have found that more ethanol is produced from glucose than from fructose, but in this work ethanol was produced at approximately equal rates in doughs made with 2% glucose, fructose or sucrose.

Yeast activity in dough is inhibited by high osmotic pressure brought about by either low water content or high solute (salt or sugar) content (section 1.3). In doughs prepared here, the optimal glucose concentration for rapid and sustained gas production was between 1.0% and 2.0%. Gas production continued once glucose was exhausted, and a post-maximal plateau in gassing rate may have been due to yeast adaptation to utilise maltose (Koch et al. 1954; Suomalainen et al. 1972).

Subsequent experimental work used a formulation containing 2% glucose as the sugar source.

The toxic effect of ethanol on micro-organisms is well-documented, but *Saccharomyces cerevisiae* tolerates relatively high levels of ethanol (see section 1.9). Production of ethanol and consumption of glucose were not inhibited by up to 16.9 mmol.(100g dough)$^{-1}$ (10 ml.kg^{-1}) added ethanol, the equivalent produced in a 90-minute fermentation. While experiments were not replicated enough to establish statistical rigour, it appeared that low levels of added ethanol stimulated gas production.

The flour used here contained slightly less protein than is usually recommended for frozen doughs, but this is unlikely to have affected the fermentation rate of yeast.

6. YEAST METABOLITE DYNAMICS IN FRESH DOUGH

6.1. Introduction

In the previous section dough formulation was optimised so that fermentation was not limited by a lack or sugar or inhibited by an excess of sugar. It was then necessary to quantify all the relevant sources of sugar in dough. Published methods for extracting sugars and ethanol from dough were unsuitable, so a new method was developed and validated.

The quantitative relationships between yeast substrate uptake and byproduct excretion during fermentation would reveal the extent of oxidative metabolism operating in dough. These molar ratios were also needed for mathematical modelling.

6.2. Extraction of sugars and ethanol from dough

Nine methods were found for quantifying low molecular weight organic solutes in dough and related products, and suitability for extracting ethanol and sugars from fermenting yeasted dough was assessed. The AACC method “Simple sugars in cereal products by HPLC” (American Association of Cereal Chemists 1995) was not suitable because it would not eliminate yeast activity. Five methods that used hot ethanolic solvents to extract sugars (Koch et al. 1954; Tang et al. 1972; Varo et al. 1979; Potus et al. 1994; Langemeier and Rogers 1995) would obscure ethanol in the sample. The method of Rouzaud and Martinez-Anaya (1993) was complex, and simple aqueous extractions with one part dough to ten parts water had proven suitable for extraction of ethanol and sugars (Saalfeld and Freund 1998; Lefebvre et al. 2002).

The method used here was based on the aqueous extraction methods of Saalfeld and Freund (1998) and Lefebvre et al. (2002). Preliminary trials indicated that homogenising a piece of dough in water did not break up the sample very well. For this reason (and to rapidly quench yeast activity) dough was frozen in liquid nitrogen and shattered into a powder prior to extraction in water at a ratio of 1 part dough to 10 parts water. Several filters with different pore sizes were trialled and a 0.8 µm pore size was chosen because it would remove yeast cells but was not fouled by solids too quickly.

Quantification of sugars and ethanol in dough extract has been performed with high-performance liquid chromatography (HPLC) (Varo et al. 1979; Rouzaud and Martinez-Anaya 1993; Potus et al. 1994; Langemeier and Rogers 1995; Lefebvre et al. 2002)

and enzymatic assays (Saalfeld and Freund 1998; Lefebvre et al. 2002). When extracts are analysed by HPLC protein must be removed to protect the column.

Equipment for automation of the enzymatic assay was available in the Massey University Nutrition laboratory. Dough extracts are sometimes deproteinised before enzymatic assays, but this was considered unnecessary for the automated assay, which involved dilutions of 3.7-fold for ethanol and more than 50-fold for sugars. Initial results showed good recovery of sugars and ethanol from spiked doughs, suggesting that protein was not interfering with the assay.

6.2.1 Recovery of solutes from spiked doughs

Extraction efficiency was measured by spiking unyeasted doughs with known quantities of glucose, fructose, maltose, sucrose or ethanol. The endogenous sugars originating from raw materials were measured in control doughs without added chemicals. Recovery data are listed in Appendix A1 and summarised in Table 6.1.

Table 6.1. Recovery of sugars and ethanol from spiked doughs.

solute	*spiked amount*	*% recovery*[A]	*n*[B]
ethanol	2 ml.kg^{-1}	101.0 ± 3.7[C]	6
	5 ml.kg^{-1}	109.9 ± 7.0	6
	10 ml.kg^{-1}	98.6 ± 9.5	14
	25 ml.kg^{-1}	93.1 ± 2.8	8
	pooled	99.7 ± 8.8	34
glucose	5 g.kg^{-1}	88.3 ± 2.4	6
	10 g.kg^{-1}	96.5 ± 5.6	6
	pooled	92.4 ± 5.9	12
fructose	5 g.kg^{-1}	98.2 ± 4.1	6
	10 g.kg^{-1}	102.8 ± 2.4	6
	pooled	100.5 ± 4.0	12
sucrose	5 g.kg^{-1}	105.6 ± 10.0	6
	10 g.kg^{-1}	107.6 ± 4.5	6
	pooled	106.6 ± 7.5	12
maltose	5 g.kg^{-1}	87.9 ± 16.3	4
	10 g.kg^{-1}	54.0 ± 27.5	4
	pooled	71.0 ± 27.7	8

Doughs were frozen in liquid nitrogen, ground and homogenised in water. Homogenate was filtered (0.8 µm) and assayed for solutes.

A. amount recovered (corrected for endogenous metabolite in unspiked doughs) divided by the amount added and multiplied by 100

B. number of replicate assays

C. mean ± standard deviation

Recovery of all solutes was consistently high, with the exception of one of the two extracts from the dough with 10 $g.kg^{-1}$ added maltose. These results (37.0 and 23.1% recovery) were probably a result of an error in analysis and the second extract gave better results (81.2 and 63.9% recovery). The maltose assay calculation required prior measurement of sucrose and glucose levels (section 1.4.7), which contributed to the high standard deviation.

6.2.2 Extraction with heating

When extracts were re-assayed after several hours at room temperature (20˚C) in COBAS sample racks, higher glucose concentrations were often recorded. Samples remained in racks for up to seven hours when assay calibration had to be repeated or when several different assays were run in sequence.

There were no consistent changes in assay results from control sera, and it was thought that the increase in glucose could result from chemical or enzymatic reactions in dough extracts. Other workers have extracted dough in aqueous ethanol at 80˚C for up to an hour (Tang et al. 1972; Varo et al. 1979; Potus et al. 1994) to enhance sugar recovery, kill yeast cells and denature enzymes such as α-amylases. A similar heating step was added to the extraction procedure to denature soluble enzymes in the extract.

Samples of frozen ground dough that had been stored at -80˚C were extracted as before, i.e. homogenised in water at room temperature and filtered. In addition, filtrate was heated at 85˚C for 1h, cooled on ice for 10 minutes and centrifuged. The supernatant was pipetted into a new tube and analysed for sugars. Heating resulted in the precipitation of a small amount of gelatinous white material. Extracts to be assayed for ethanol were never heated so that evaporative losses were minimised.

Table 6.2. Recovery of sugars from spiked doughs when extracts were heated at 85˚C for 1 h prior to analysis.

solute	*spiked amount*	*% recovery*[A]	*n*[B]
glucose	5 $g.kg^{-1}$	92.1 ± 3.3[C]	4.0
	10 $g.kg^{-1}$	96.2 ± 1.6	4.0
	pooled	94.1 ± 3.3	8.0
fructose	5 $g.kg^{-1}$	114.7 ± 7.9	4.0
	10 $g.kg^{-1}$	110.8 ± 4.7	4.0
	pooled	112.7 ± 6.3	8.0
sucrose	5 $g.kg^{-1}$	97.3 ± 10.2	4.0
	10 $g.kg^{-1}$	96.2 ± 14.9	4.0
	pooled	96.7 ± 11.8	8.0
maltose	5 $g.kg^{-1}$	99.0 ± 19.9	4.0
	10 $g.kg^{-1}$	85.3 ± 11.9	4.0
	pooled	92.2 ± 16.8	8.0

Doughs were frozen in liquid nitrogen, ground and homogenised in water. Homogenate was filtered (0.8 µm) and heated at 85˚C for 1 h then cooled on ice and centrifuged. Supernatant was assayed for solutes.

A. amount recovered (corrected for endogenous metabolite in unspiked doughs) divided by the amount added and multiplied by 100

B. number of replicates - spiked doughs were extracted twice and analysed in duplicate

C. mean ± standard deviation

The control for these experiments was a dough made without yeast or added sugar, frozen, ground and stored at -80˚C as part of an earlier experiment. It was later found that this dough was made with a different batch of flour from the spiked doughs, and there may have been differences in endogenous sugars between the two batches.

Recovery of spiked analytes from heated extracts was consistently high (Table 6.2). Maltose recovery was improved by heating. Apparently there was more endogenous fructose in the spiked dough than in the control, resulting in recoveries above 100%.

Sugar concentrations in heated and unheated extracts of the same spiked doughs were analysed for statistical differences (Table 6.3).

Table 6.3. Sugar concentrations in heated and unheated extracts from defined doughs.

sugar tested	*added sugar*	*total recovered sugar*		*statistical significance*[B]
	g.(kg dough)$^{-1}$	*mmol.(100g dough)*$^{-1}$		
		unheated	*heated*[C]	
glucose	5	2.77 ± 0.02[A]	2.91 ± 0.10	*
	10	5.85 ± 0.27	5.80 ± 0.10	ns
fructose	5	3.26 ± 0.10	3.43 ± 0.23	ns
	10	6.40 ± 0.09	6.38 ± 0.50	ns
sucrose	5	3.03 ± 0.03	3.45 ± 0.15	*
	10	4.34 ± 0.11	4.97 ± 0.44	*
maltose	5	1.27 ± 0.23	1.43 ± 0.29	ns
	10	1.55 ± 0.79	2.45 ± 0.34	ns

A. mean ± standard deviation of six replicates (unheated) or four replicates (heated)

B. T-test with null hypothesis that mean concentrations in heated and unheated extracts were equal. 'ns' - no significant difference at 95% confidence, '*' – significant difference at 95% confidence.

C. dough extracts heated at 85˚C for 1 h prior to analysis

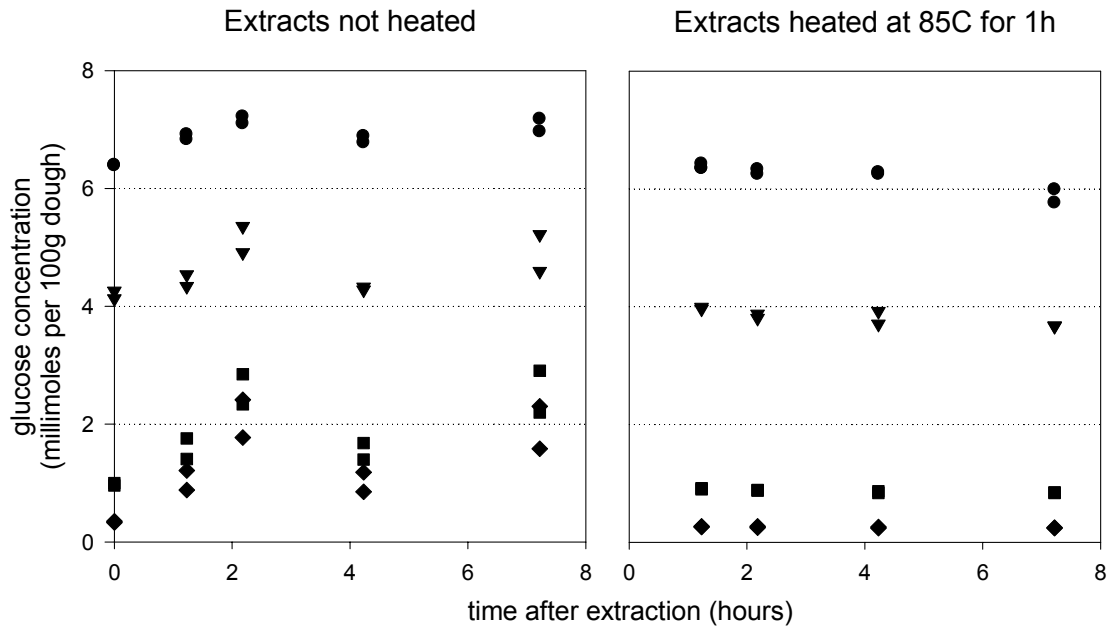
Maltose and fructose concentrations were unaffected by heating, and glucose was significantly different in one of the two spiked doughs. Heated extracts contained significantly more sucrose than unheated extracts. A possible explanation is that invertases in the extracts were denatured by heating and sucrose was hydrolysed to a lesser extent. However this was not borne out by any corresponding increases in glucose and fructose.

6.2.3 Extract stability at room temperature

Changes in glucose concentration in extracts stored at room temperature (20˚C) were measured using extracts from doughs made with 2% sucrose and fermented 0, 60, 120 or 180 minutes at 30˚C. Storage time ranged from 0 to 7 hours.

Frozen, ground dough samples were homogenised in water and filtered then divided into two aliquots. One was assayed immediately (0 hours storage) and the other heated at 85˚C for 1 hour. Both heated and unheated extracts were assayed on completion of heating, which was 1 hour and 14 minutes after extraction. Both sets of extracts in COBAS sample tubes were stored at room temperature (20˚C) and re-assayed in duplicate over the next 6 hours.

Figure 6.1. Changes in glucose concentration in dough extracts during storage at room temperature.



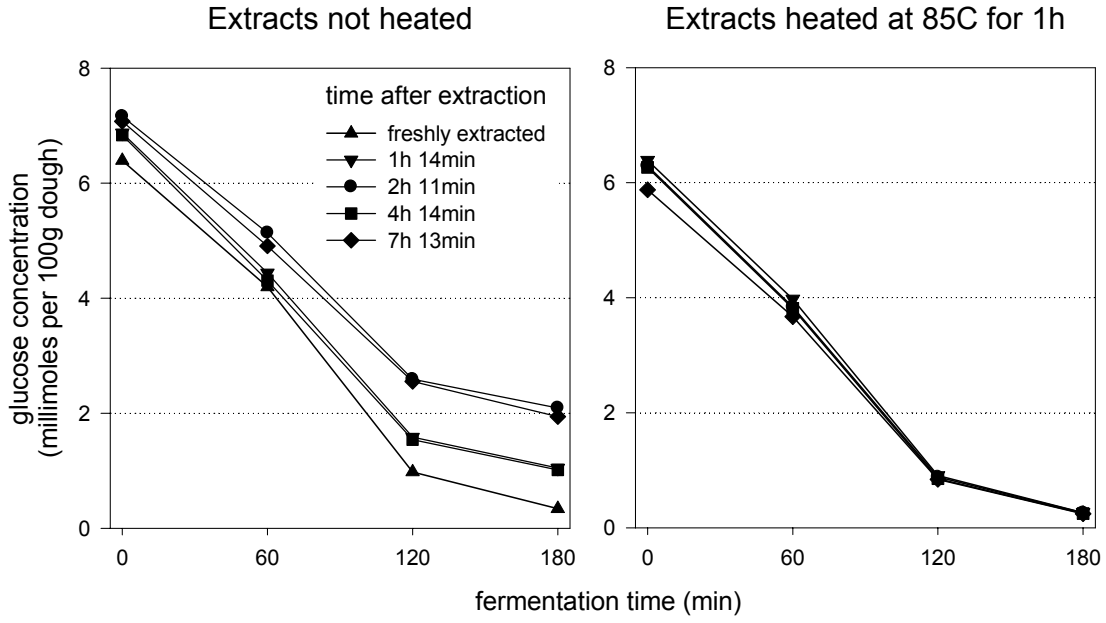
Extracts were from dough made with 2% sucrose and fermented 0-180 minutes at 30˚C. Extracts were held at room temperature (20˚C) in analyser tubes for the indicated time after extraction. Each point is a single assay result.

Glucose concentrations in heated and unheated extracts are plotted in Figure 6.1 and listed in Appendix A2. In unheated extracts there was a 1-2 mmol.L^{-1} rise in glucose concentration over the first two hours, which appeared to be independent of fermentation time.

Results were analysed using balanced analysis of variance (Appendix A2) with factors 'storage time', 'heating', and 'fermentation time'. ANOVA showed significant effects at 95% confidence for all three factors and significant interactions for 'storage time' x 'heating' and 'fermentation time' x 'heating'.

The significant 'fermentation time' effect was a result of yeast consuming sugars throughout the course of the fermentation. The significant 'storage time', 'heating' and 'storage time' x 'heating' effects indicated that glucose concentrations were changing at room temperature and that the heated and unheated extracts showed different patterns of change. The fermentation time x heating interaction indicated that the effect of heating varied with fermentation time, and this is also evident in Figure 6.2.

Figure 6.2. Impact of storage time at room temperature on the sugar concentration time course in unheated and heated extracts.



Doughs made with 2% added sucrose and fermented at 30˚C for 0-180 minutes were extracted at room temperature and extract divided into duplicate aliquots. One set was assayed for glucose immediately and after 1 to 7 hours at room temperature (20˚C). The other was heated at 85˚C for 1h, cooled and centrifuged then assayed for glucose after 1 to 6 hours at room temperature. Data points are the mean of duplicate assay results.

Heated extracts showed less variation in glucose concentration during storage than unheated extracts (Figure 6.1). When extracts were not heated the time course of sugar concentration in a fermenting dough was influenced by the duration of the delay between extraction and analysis, especially at longer fermentation times (Figure 6.2).

6.3. Endogenous sugars in flour

Flour samples from three of the batches used in this work were extracted with and without heating then assayed for glucose, fructose, sucrose and maltose (Table 6.4).

Table 6.4. Endogenous sugar levels in flour.

batch[A]	glucose			fructose			sucrose			maltose		
	mmol.(100g flour)$^{-1}$											
	mean	SD[B]		mean	SD		mean	SD		mean	SD	
UNHEATED												
1	0.33	0.08	a[C]	0.18	0.09	-	2.56	0.18	b	3.51	0.40	a
2	0.24	0.03	a	nd[D]	-	-	3.03	0.17	a	3.61	0.32	a
3	0.21	0.06	b	nd	-	-	2.88	0.14	a	3.79	0.47	a
pooled	0.26	0.08		0.18	0.09		2.82	0.26		3.63	0.39	
HEATED												
1	0.32	0.02	a	0.17	0.05	-	2.43	0.26	b	3.00	0.32	a
2	0.18	0.04	b	nd	-	-	2.95	0.19	a	3.06	0.33	a
3	0.19	0.05	b	nd	-	-	2.85	0.11	a	3.31	0.32	a
pooled	0.23	0.08		0.17	0.05		2.74	0.30		3.12	0.33	

Extractions were carried out on 30 March 2005. Each sample was extracted three times, both with and without heating then assayed twice.

A. batch 1 was packed on 22/8/03, batch 2 on 6/5/04 and batch 3 on 5/8/04

B. standard deviation

C. lower case letters indicate significant differences (95% confidence) between batches for a given extraction method

D. not detected

Flours were stored in the original multi-walled paper bags at room temperature in a laboratory cupboard, but chemical or enzymatic reactions may nevertheless have occurred. This would explain the detection of fructose in flour from batch 1 in conjunction with somewhat higher glucose and significantly lower sucrose than the other two batches. There were no significant differences in maltose, which suggested that amylase activity during storage was negligible.

6.4. Sugar reactions during dough mixing

Mixing hydrates dry ingredients, develops the structure of gluten and distributes yeast cells throughout the dough. Conditions are suitable for amylases and yeast enzymes to become active and to be brought into contact with sugars and oligosaccharides, changing the sugar profile of doughs. This section quantifies the changes in the sugar profiles of yeasted and unyeasted doughs during 18 minutes of mixing.

The amount of sugar supplied by ingredients at the start of mixing was estimated from endogenous flour sugars (Table 6.4) and the amount of added sugar in the formulation. Table 6.5 shows a comparison between the sugars supplied by ingredients and sugars in yeasted and unyeasted 2% sucrose doughs immediately after mixing.

Yeasted doughs contained significantly more glucose and fructose and less sucrose than unyeasted doughs, in agreement with reports of rapid sucrose hydrolysis in yeasted doughs.

Hydrolysis of sucrose yields equimolar amounts of glucose and fructose. In 2% sucrose doughs (Table 6.5) the molar decrease in sucrose with mixing, i.e. sucrose in ingredients minus sucrose in yeasted dough at the end of mixing, corresponded with the molar increase in glucose to within 0.24 mmol.(100g dough)$^{-1}$. However the increase in fructose exceeded the drop in sucrose by 2.95 and 3.36 mmol.(100g dough)$^{-1}$ (unheated and heated respectively).

Unyeasted doughs extracted without heating contained significantly higher fructose than ingredients, but the difference was not significant when extracts were heated. More sucrose was recovered from unyeasted doughs when extracts were heated, as with spiked doughs (section 6.2), but heating had no effect on sucrose recovery from ingredients or yeasted doughs. Maltose was highest in yeasted doughs for reasons that are unclear.

A substantial amount of fructose was apparently liberated during mixing in yeasted doughs, probably due to the action of yeast invertases on fructans.

Table 6.5. Sugars in ingredients and freshly-mixed doughs with 2% added sucrose.

		glucose				fructose				sucrose				maltose			
		mmol.(100g dough)$^{-1}$				mmol.(100g dough)$^{-1}$				mmol.(100g dough)$^{-1}$				mmol.(100g dough)$^{-1}$			
		mean	SD[A]	n[B]		mean	SD	n		mean	SD	n		mean	SD	n	
unheated	ingredients	0.16	0.05	18	c[C]	0.11	0.05	18	c	7.54	0.15	18	b	2.18	0.24	18	bc
	unyeasted dough	0.87	0.57	6	b	1.87	1.30	6	b	6.48	0.49	6	c	1.75	0.57	4	c
	yeasted dough	6.83	0.48	8	a	9.93	0.57	8	a	0.67	0.54	8	d	2.47	0.49	6	b
heated	ingredients	0.14	0.05	18	c	0.10	0.03	18	c	7.49	0.18	18	b	1.87	0.20	18	c
	unyeasted dough	0.16	0.08	6	c	0.25	0.04	6	c	8.06	0.43	6	a	2.31	0.53	4	bc
	yeasted dough	6.46	0.67	6	a	10.02	1.00	6	a	0.93	0.54	6	d	3.18	0.60	4	a

A. standard deviation

B. number of replicates

C. lower case letters indicate significant differences down columns at 95% confidence

In separate experiments doughs were made with 2% added glucose or fructose instead of sucrose. Adding 2% w/w of either hexose would theoretically increase its concentration in the dough by 11.11 mmol.(100g dough)$^{-1}$ above the endogenous level supplied by flour.

By this reasoning the dough with 2% added glucose was expected to contain up to 11.27 mmol.(100g dough)$^{-1}$. The dough was extracted without heating at the completion of mixing and found to contain 10.43 mmol.(100g dough)$^{-1}$ glucose, a difference of 0.85 mmol.(100g dough)$^{-1}$ from the theoretical figure. This probably indicates consumption of glucose during mixing.

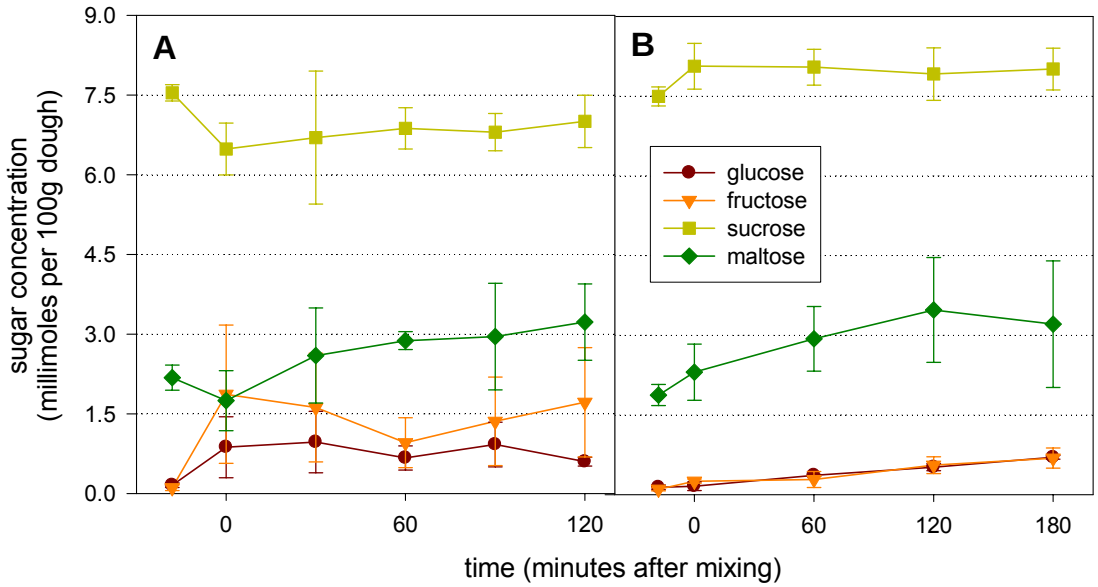
The dough with 2% added fructose was expected to contain up to 11.22 mmol.(100g dough)$^{-1}$ fructose. However 13.95 mmol.(100g dough)$^{-1}$ was recovered by extraction without heating immediately after mixing, a difference of 2.73 mmol.(100g dough)$^{-1}$. Fructose appeared to be liberated during mixing of dough with 2% added fructose, as with the 2% sucrose dough.

6.5. Sugar dynamics in unyeasted dough

In unyeasted dough only the activity of endogenous and microbial enzymes will affect sugars (the red pathways in Figure 1.6). Unyeasted doughs were extracted with or without heating and assayed for sugars (Figure 6.3).

Maltose increased slowly up to 90-120 minutes then was unchanged or declined slightly. Sucrose was lower in unheated extracts, but changed little throughout the fermentation. Maltose and sucrose results are similar to other reports (Suomalainen et al. 1972; Seppi 1984; Potus et al. 1994).

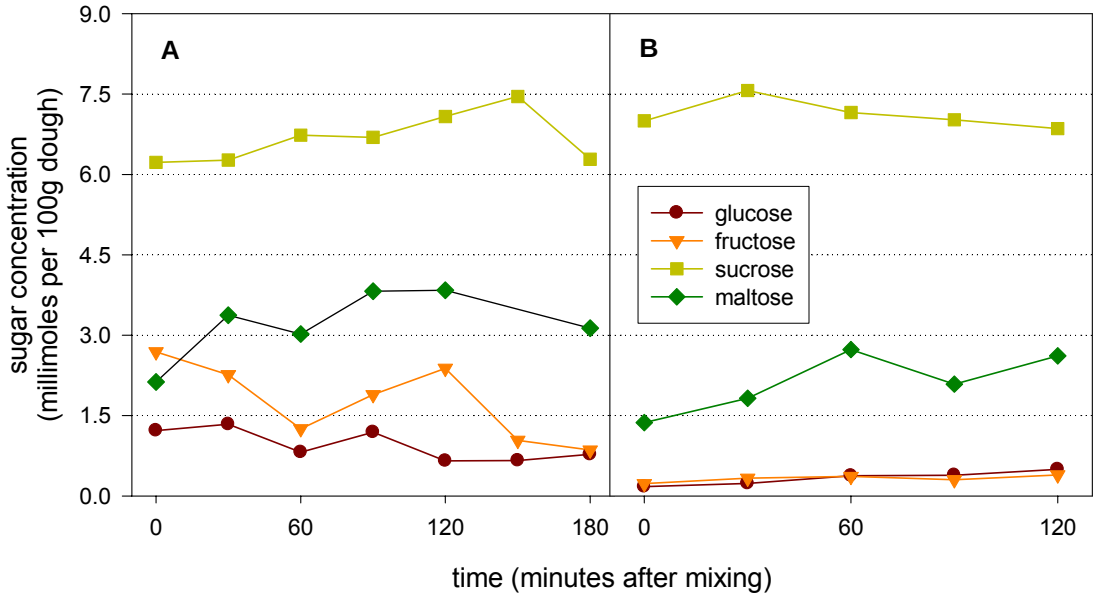
Figure 6.3. Sugars in unyeasted doughs made with 2% added sucrose fermenting at 30˚C.



A: extracted without heating; B: extracts heated at 85˚C for 1 h prior to analysis. The data points to the left of zero time are estimated from the sugar levels in flour. Vertical bars are standard deviations of duplicate assays of at least two doughs.

Glucose and fructose were higher and more variable in the unheated extracts. Unheated extracts from one of the duplicate 2% sucrose doughs were assayed for sucrose on 20 October 2004 and a delay due to equipment malfunction meant that samples could not be assayed for glucose and fructose on the same day. They were frozen and stored at -20˚C until 28 October then thawed and assayed for glucose and fructose.

Figure 6.4. Sugars in two replicate unyeasted doughs made with 2% added sucrose fermenting at 30˚C, extracted without heating.



A. dough extracts were assayed for sucrose then frozen at -20˚C until 28 October when they were thawed and assayed for glucose and fructose. B. extracts assayed for glucose, sucrose and fructose on the same day. Points are the mean of at least 2 assay results.

Results were quite different from the second replicate, extracts of which were assayed for glucose, fructose and sucrose on the same day (Figure 6.4). The delay and freeze-thaw cycle apparently caused fluctuations in glucose and fructose results not seen in extracts of the second replicate (Figure 6.4B). Sucrose and maltose calculations were also affected because they involve subtracting the glucose result. In the second replicate dough glucose and fructose results were very similar to heated dough extracts (Figure 6.3B) and reports by other workers (Suomalainen et al. 1972; Seppi 1984; Potus et al. 1994).

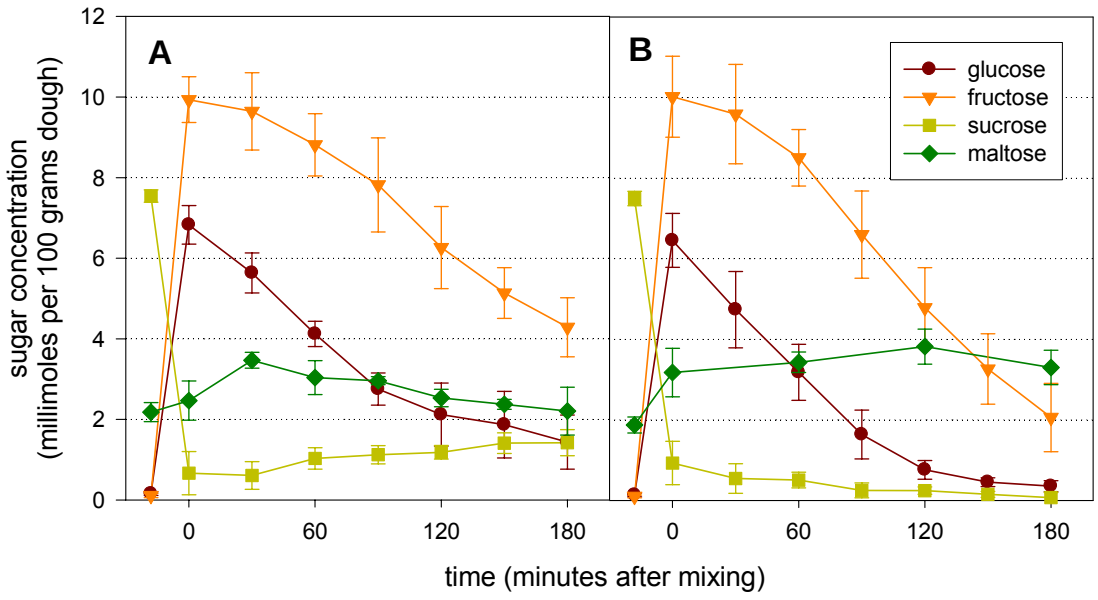
6.6. Sugar dynamics in yeasted dough

Doughs made with 2% sucrose and 2% yeast were extracted and assayed for sugars with or without heating (Figure 6.5). Sucrose fell dramatically with mixing in both sets of extracts, as previously reported (Koch et al. 1954; Seppi 1984; Potus et al. 1994).

The initial decline in sucrose coincided with a jump in fructose and glucose. The molar increase in fructose was more than the molar decrease in sucrose, indicating an additional source of fructose (see section 6.4). Glucose initially declined faster than fructose, then as glucose became depleted fructose was consumed more rapidly. This is in agreement with reports that *Saccharomyces cerevisiae* takes up glucose in preference to fructose (Koch et al. 1954; Orlowski and Barford 1987; D'Amore et al. 1989).

In unheated extracts glucose plateaued at 2-3 mmol.(100g dough)$^{-1}$ after 90 minutes of fermentation, and sucrose increased slowly after the initial rapid drop. These features have not been reported before, and may be artifacts of the extraction procedure that were eliminated by heating.

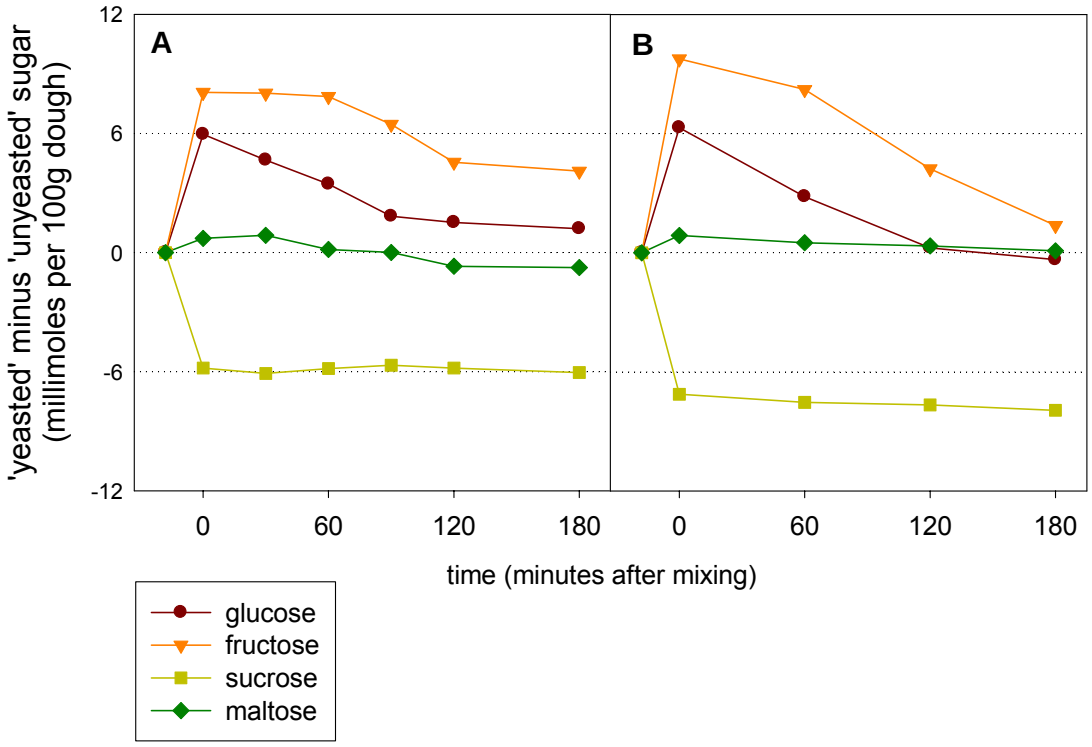
Figure 6.5. Sugars in yeasted doughs made with 2% sucrose fermenting at 30˚C.



A: extracted without heating; B: extracts heated at 85˚C for 1 h prior to analysis. The data points to the left of zero time are estimated from the sugar levels in flour. Data points are the mean of duplicate assays on extracts of three independent doughs. Vertical bars are standard deviations.

The effect of yeast on the sugar profile of dough can be seen by calculating the difference between sugars in yeasted and unyeasted doughs (Figure 6.6). Sucrose hydrolysis was complete by the end of mixing and maltose was unchanged by yeast. Heating emphasised the drop in glucose and fructose due to the presence of yeast.

Figure 6.6. The 'yeasted minus unyeasted' difference in sugar levels for doughs made with 2% sucrose and fermented at 30˚C.



A: extracted without heating; B: extracts heated at 85˚C for 1 h prior to analysis. Data points are the difference in means of duplicate assays of 3 independent doughs.

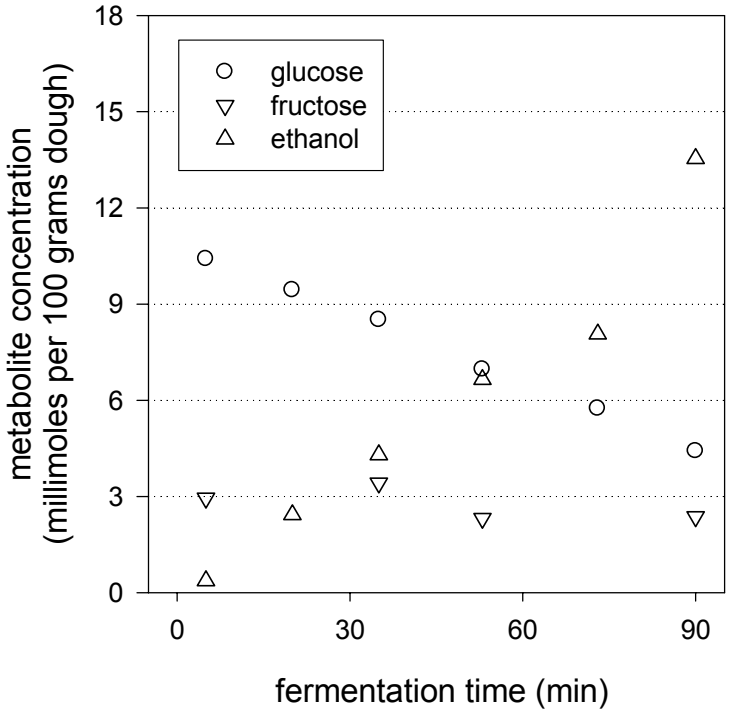
6.7. Ethanol yield from monosaccharides

Yeast cells in broth culture take up glucose faster than fructose when both are present, but they are consumed at similar rates in the absence of the other (Orlowski and Barford 1987). This section compares glucose and fructose uptake rates in dough and the yield of ethanol from each. It is difficult to eliminate either monosaccharide from dough because of endogenous levels in flour, but a large excess of one sugar can be created by adding exogenous sugar.

In dough made with 2% added glucose (11.1 mmol.(100g dough)$^{-1}$) there was a consistent linear decline in glucose throughout fermentation (Figure 6.7). Endogenous fructose stayed approximately constant at 3 mmol.(100g dough)$^{-1}$. Glucose data were well-fitted by linear regression analysis (r-squared 98.7%) which gave an intercept of 10.85 ± 0.09 mmol.(100g dough)$^{-1}$ and slope of -0.0710 ± 0.0017 mmol.(100g dough)$^{-1}$.minute^{-1}.

During the first 75 minutes of fermentation, ethanol accumulated at a constant rate of 0.129 ± 0.001 mmol.(100g dough)$^{-1}$.minute^{-1} (r-squared 99.9%).

Figure 6.7. Glucose, fructose and ethanol in dough made with 2% glucose and 2% yeast fermenting at 30˚C.



Data points are the mean of duplicate assays but duplicates were not averaged for regression analysis.

In doughs with 2% added fructose, one replicate showed an increase in fructose in the first 30 minutes and the other a slight decline (Figure 6.8). Between 30 and 90 minutes fructose fell at a rate of 0.0787 ± 0.0067 mmol.(100g dough)$^{-1}$.min^{-1} (r-squared 89.6%) and ethanol increased at 0.147 ± 0.009 mmol.(100g dough)$^{-1}$.min^{-1} (r-squared 96.8%). Glucose changed little during the 90-minute fermentation.

Molar yields of ethanol from monosaccharides were calculated by dividing slopes:

$$\frac{moles\ ethanol}{mole\ sugar} = \frac{moles\ ethanol \cdot 100g^{-1} \cdot minute^{-1}}{moles\ sugar \cdot 100g^{-1} \cdot minute^{-1}} \qquad 6.1$$

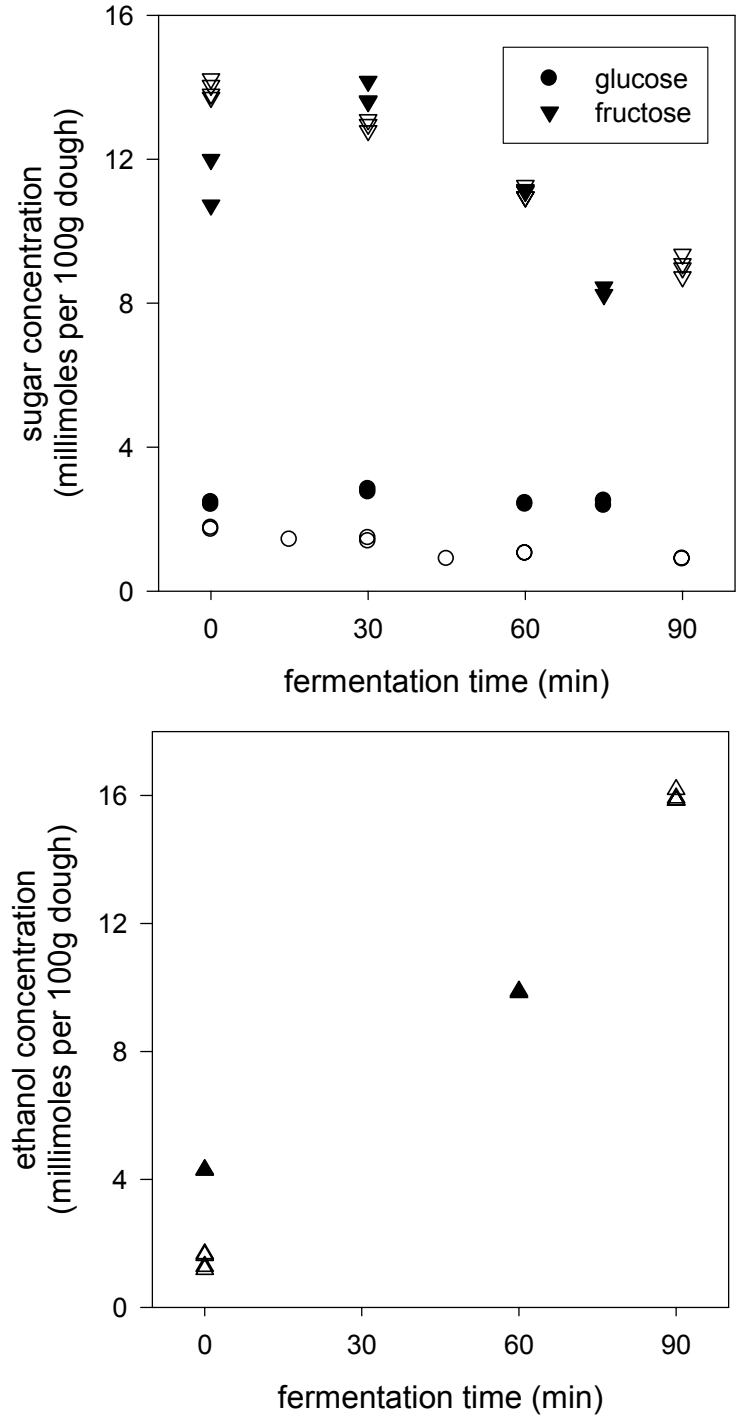
The yields were 1.82 and 1.87 for glucose and fructose respectively, which are within the range of reported yields (Table 6.6).

Table 6.6. Molar yields of ethanol from glucose in published *Saccharomyces cerevisiae* fermentation studies.

yield	*study*	*comment*
1.49	Barford and Hall (1981)	direct measurement
1.66	Peringer et al. (1974)	direct measurement
1.42 1.48[A]	Wang et al. (2004)	direct measurement
1.88	Sweere et al. (1988)	mathematical model
1.79	Lafforgue-Delorme et al. (1994)	mathematical model
1.68	Lee et al. (1995)	mathematical model
1.60	Smogrovicova et al. (2001)	mathematical model

A. substrate was fructose

Figure 6.8. Glucose, fructose (top) and ethanol (bottom) in doughs made with 2% fructose and fermented at 30˚C.



Open and closed symbols indicate replicates. Each data point is a single assay result.

6.8. Sugar – ethanol mass balance

The only quantitatively important sugars in dough are glucose, fructose, sucrose and maltose, all of which *Saccharomyces cerevisiae* will consume. Maltose uptake is repressed by glucose and fructose (catabolite repression), an effect observable at monosaccharide concentrations as low as 25 $mmol.L^{-1}$ (Lovgren and Hautera 1977). Maltose in dough declines only once glucose and fructose are depleted (Koch et al. 1954), e.g. in the later stages of sponge and dough fermentations (Tang et al. 1972).

Maltose was not consumed during the 3-hour straight dough fermentations carried out in this work (section 6.6), so only glucose, fructose and sucrose need be considered as yeast substrates here.

It is convenient to express glucose and fructose from endogenous and exogenous sources (including sucrose hydrolysis) as the composite quantity 'total available monosaccharides' (TAM). TAM was calculated as the sum of molar quantities of glucose and fructose plus twice the molar quantity of sucrose, with units of millimoles per 100g dough. It gave a measure of how fast the pool of readily-metabolisable sugars in dough was depleted.

Three doughs made with 2% sucrose were divided into 50 g subsamples, which were placed in a temperature-controlled room at 30˚C. Samples were taken out and extracted every 30 minutes for 180 minutes, and extracts were assayed in duplicate for glucose, fructose, sucrose and ethanol.

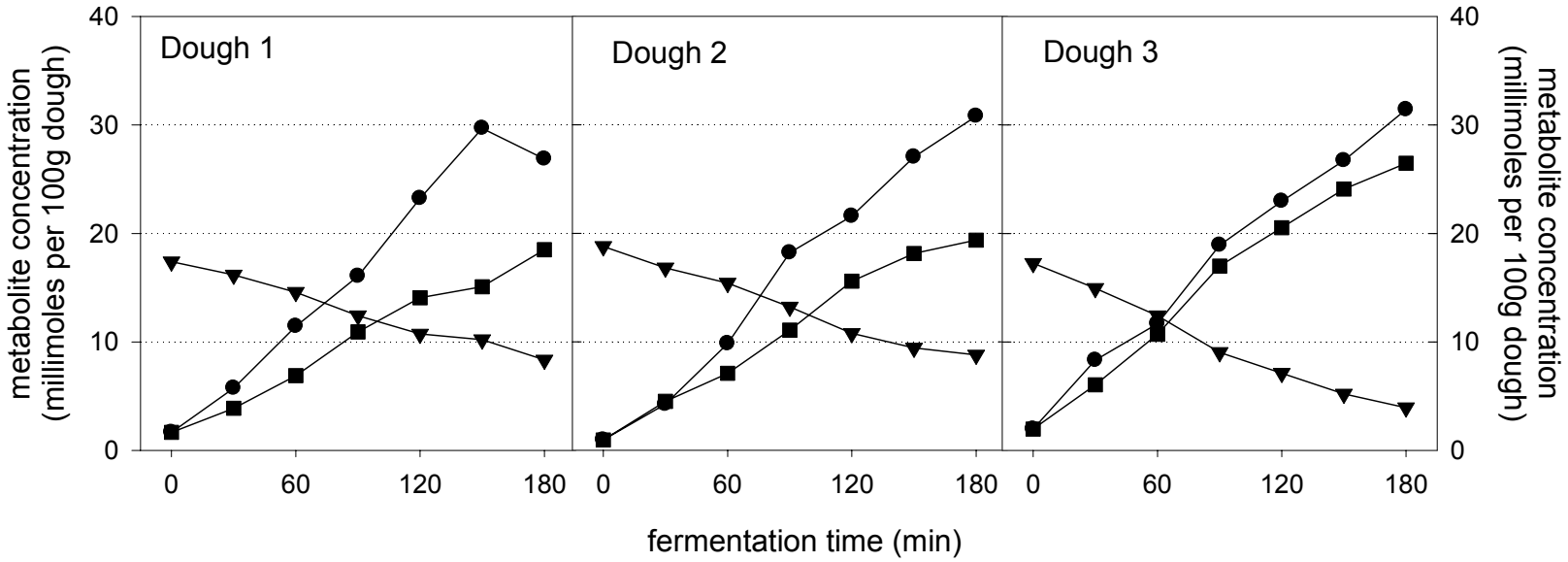
Ethanol was predicted from the consumption of sugars using yields calculated in section 6.7, and predictions are compared with measured ethanol in Figure 6.9.

Extracts were not heated at first but when it was found that heating improved extract stability, samples of ground dough stored at -80˚C were re-extracted with heating at 85˚C for 1h and extracts were assayed for sugars. Ethanol measurements on unheated extracts were not repeated with heated extracts. Ethanol predictions from sugars in heated extracts are compared with measured ethanol from unheated extracts in Figure 6.10.

During the 3-hour fermentation TAM fell 10-15 mmol.(100g dough)$^{-1}$ in unheated extracts. Ethanol predictions varied from good (Figure 6.9, Dough 3) to poor (Figure 6.9, Dough 1), probably a reflection of fluctuations in sugar concentration occurring while extracts were at room temperature before being assayed (section 6.2.3).

TAM fell almost 20 mmol.(100g dough)$^{-1}$ in heated extracts and predictions were consistently good (Figure 6.10). The close match during three hours of fermentation confirmed the accuracy of ethanol yields and ruled out other sugar sources.

Figure 6.9. Measured ethanol and ethanol predictions from sugar levels in unheated dough extracts.

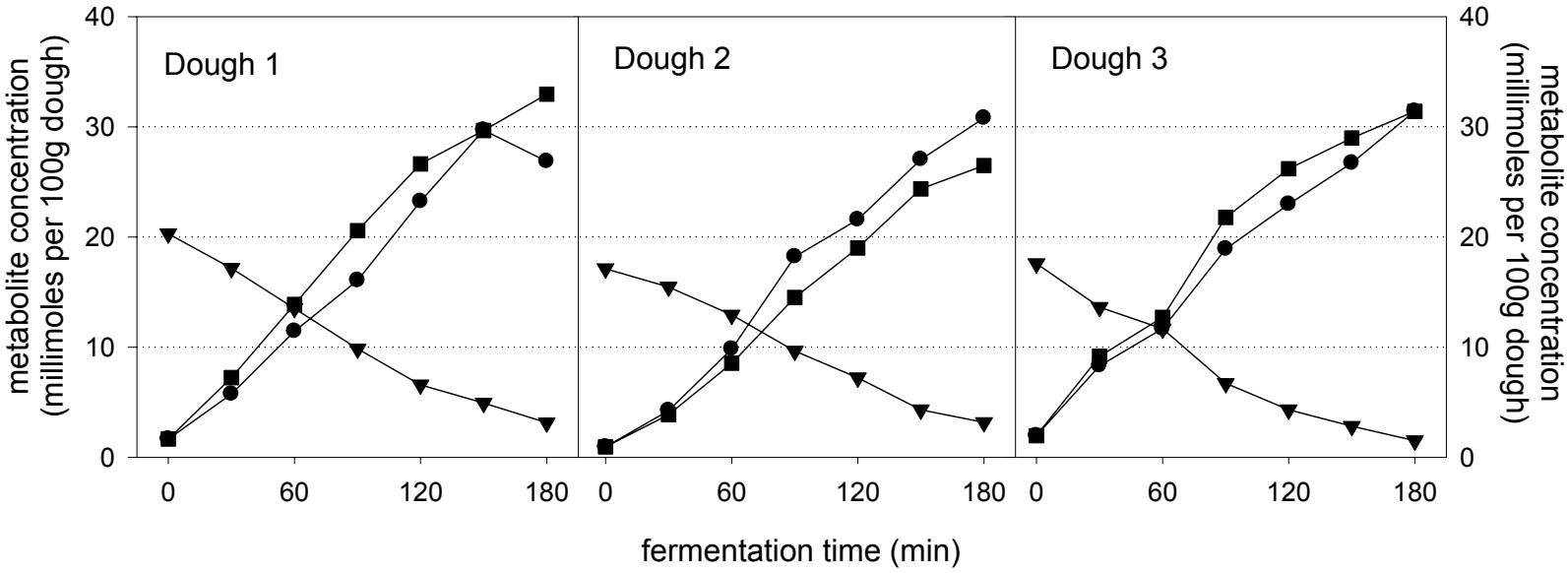


Doughs were made with 2% added sucrose and fermented at 30˚C for 0-180 minutes then extracted. Dough extracts were analysed for sugars and ethanol without prior heating. Ethanol was predicted from the consumption of glucose, fructose and sucrose using experimentally-determined molar yields.

Total available monosaccharides (TAM) was calculated as (moles glucose) + (moles fructose) + 2 x (moles sucrose). Data are the mean of duplicate assays on samples extracted once or twice.

Doughs 1, 2 and 3 are independent replicates.

Figure 6.10. Measured ethanol and ethanol predictions from sugar levels in heated extracts.



Doughs were made with 2% added sucrose and fermented at 30˚C for 0-180 minutes then extracted. Dough extracts for sugar analysis were heated at 85˚C for 1 h prior to analysis but extracts for ethanol analysis were not heated. Ethanol was predicted from the consumption of glucose, fructose and sucrose using experimentally-determined molar yields

Total available monosaccharides (TAM) was calculated as (moles glucose) + (moles fructose) + 2 x (moles sucrose). Data are the mean of duplicate assays on samples extracted once or twice.

Doughs 1, 2 and 3 are independent replicates.

6.9. Carbon dioxide – ethanol mass balance

When a *Saccharomyces cerevisiae* cell catabolises one molecule of hexose sugar via reductive metabolism (anaerobic glycolysis), two molecules each of ethanol and carbon dioxide are produced. Oxidative metabolism produces six molecules of CO_2 per molecule of sugar and substantially more ATP than reductive metabolism but no ethanol (equations 1.10 and 1.11). Sugar is consumed more slowly in aerobic fermentations because of the higher energy yield.

No published literature has reported experimental determination of dissolved oxygen in dough or investigated whether yeast metabolism might include an oxidative component. The accumulation of ethanol and carbon dioxide indicate that reductive metabolism is operating, but a component of oxidative metabolism cannot be ruled out on the basis of current evidence. This section shows a quantitative comparison of ethanol and carbon dioxide in fermenting doughs and discusses the evidence for reductive and oxido-reductive metabolism.

Much of the CO_2 produced during the initial stage of post-mixing fermentation dissolves in the liquid phase of dough rather than evaporating into gas cells entrained during mixing (Hibberd and Parker 1976; Bloksma 1990a; Chiotellis and Campbell 2003b). Gas cells are small and internal pressure correspondingly high, since the pressure difference between convex and concave sides of a gas cell varies inversely with radius according to the Laplace equation (Atkins and De Paula 2002):

$$p_{in} - p_{out} = \frac{2\gamma}{r} \qquad 6.2$$

p_{in}	pressure at the concave side of a curved interface	[$N.m^{-2}$]
p_{out}	pressure at the convex side of a curved interface	[$N.m^{-2}$]
γ	surface tension	[$N.m^{-1}$]
r	interface radius of curvature	[m]

Devices such as the Risograph that record the increase in pressure in an airtight container of fermenting dough measure evolved gaseous carbon dioxide but not CO_2 dissolved in the dough (Hibberd and Parker 1976). The pressure increase originates from evaporation of dissolved CO_2, which is slow while the liquid phase is sub-saturated.

The sub-saturated stage may be shortened if there is already some CO_2 dissolved in the dough or prolonged if yeast cells are not yet fully active (Bloksma 1990a), e.g. if doughs are initially colder than the surrounding environment.

As the liquid phase approaches saturation, the driving force for CO_2 evaporation rises and there is a transition from slow to rapid gas cell growth (Bloksma 1990a). The increase in Risograph gassing rate is due to accelerating evaporation of dissolved CO_2 rather than accelerating CO_2 excretion from yeast cells.

Once saturation is reached, evaporation of CO_2 from the liquid phase occurs at the same rate as its excretion by fermenting yeast cells (Bloksma 1990a). Carbon dioxide evaporates from the surface of the dough so not all of it contributes to expanding gas cells (Chiotellis and Campbell 2003b). The volume of CO_2 that expands gas cells and the volume lost from the surface are both recorded by a Risograph or similar apparatus.

During the advanced stages of fermentation, carbon dioxide production may be inhibited by factors affecting yeast metabolism, e.g. depletion of sugar or accumulation of fermentation by-products.

Hibberd and Parker (1976) put the equivalent volume of dissolved CO_2 in saturated dough at 0.81 ml.(g dough)$^{-1}$, and found that the total CO_2 production rate was constant between 33 and 117 minutes after mixing. Fifty-gram doughs made here reached a Risograph gassing rate of up to 2.4 ml.min^{-1}. If carbon dioxide was produced at this rate throughout fermentation and it could be assumed that (a) no CO_2 was initially dissolved in the dough and (b) it did not evaporate until dough was saturated then it would take 17 minutes to reach 0.81 ml.(g dough)$^{-1}$. The Risograph registered an increase in pressure, i.e. carbon dioxide evaporation, as soon as readings began (5-10 minutes after mixing was completed), so one or both of these assumptions was incorrect.

There was no obvious lag in ethanol production or sugar consumption at the start of fermentation (Figure 6.9), and both continued at approximately constant rates for two hours. If metabolism was entirely reductive then carbon dioxide would also be produced at a constant rate during this time.

The absence of a lag suggests that yeast had become fully activated during mixing (18 minutes) and dividing (5 to 10 minutes). Some or all CO_2 from metabolic activity prior

to Risograph measurements would remain dissolved in the dough, so it would be partly saturated at the beginning of fermentation.

Gassing rates measured in the Risograph increased during the first 30-90 minutes of fermentation (figures 5.2, 5.3, 5.7) which suggested that CO_2 was both dissolving and evaporating. This is in agreement with indications from modelling studies (Shah et al. 1998; Chiotellis and Campbell 2003b) that bubble growth was possible in sub-saturated dough. It was not possible to quantify total carbon dioxide from Risograph data, but upper and lower bounds could be set.

To calculate the upper bound it was assumed that the maximum recorded gassing rate was equal to the total rate of CO_2 excretion, i.e. that the dough was saturated by the time the maximum gassing rate was reached. The upper bound represented CO_2 production at this rate throughout fermentation.

Gassing rate data were smoothed with a five-point moving average to dampen random noise:

$$A_n = \frac{R_{n-2} + R_{n-1} + R_n + R_{n+1} + R_{n+2}}{5} \qquad 6.3$$

Where n is the reading number, A_n is the moving average at reading n and R_{n-2} to R_{n+2} are gassing rates at readings n-2 to n+2. Moving averages were not calculated for the first or last two readings. Two or three replicate gassing rate curves were smoothed with the moving average function and maximum gassing rates from each were averaged to give an overall mean, from which the upper bound was calculated.

The Risograph gassing curve provided a lower bound that comprised both CO_2 expanding gas cells and evaporating from the surface of dough but not dissolved CO_2. Cumulative volume curves were averaged across two or three replicate subsamples. Data collected during the first ten minutes in the Risograph were discarded to avoid erroneous readings due to thermal expansion of air in the headspace of cans (see section 2).

Carbon dioxide volumes in millilitres per fifty grams of dough were converted to units of millimoles per 100 grams to facilitate comparison with ethanol levels. The molar volume of CO_2 was calculated using the virial equation of state (6.4) in the quadratic form (6.5) (Lide 2004).

$$pV_m = RT\left(1 + \frac{B}{V_m}\right) \quad (6.4)$$

$$pV_m^2 - RTV_m - RTB = 0 \quad (6.5)$$

Symbols are explained in Table 6.7. Values for R, B and P (one standard atmosphere) were obtained from Lide (2004).

Table 6.7: Parameters used to calculated the molar volume of carbon dioxide.

symbol	*description*	*value*	*unit*
P	pressure	101325	$N.m^{-2}$
V_M	molar volume	unknown	$m^3.mol^{-1}$
R	universal gas constant	8.314	$N.m.K^{-1}.mol^{-1}$
T	temperature	300	K
B	second virial coefficient	-126×10^{-6}	$m^3.mol^{-1}$

Analytical solution of equation 6.5 gave V_M = 0.02474 $m^3.mol^{-1}$. Risograph gas volumes from 50g doughs were multiplied by V_M^{-1} = 40.5 $mol.m^{-3}$ = 0.0405 $mmol.ml^{-1}$. A factor of 2 was included to give a 100g basis, bringing the conversion factor to 0.0810 $mmol.ml^{-1}.(100g\ dough)^{-1}$.

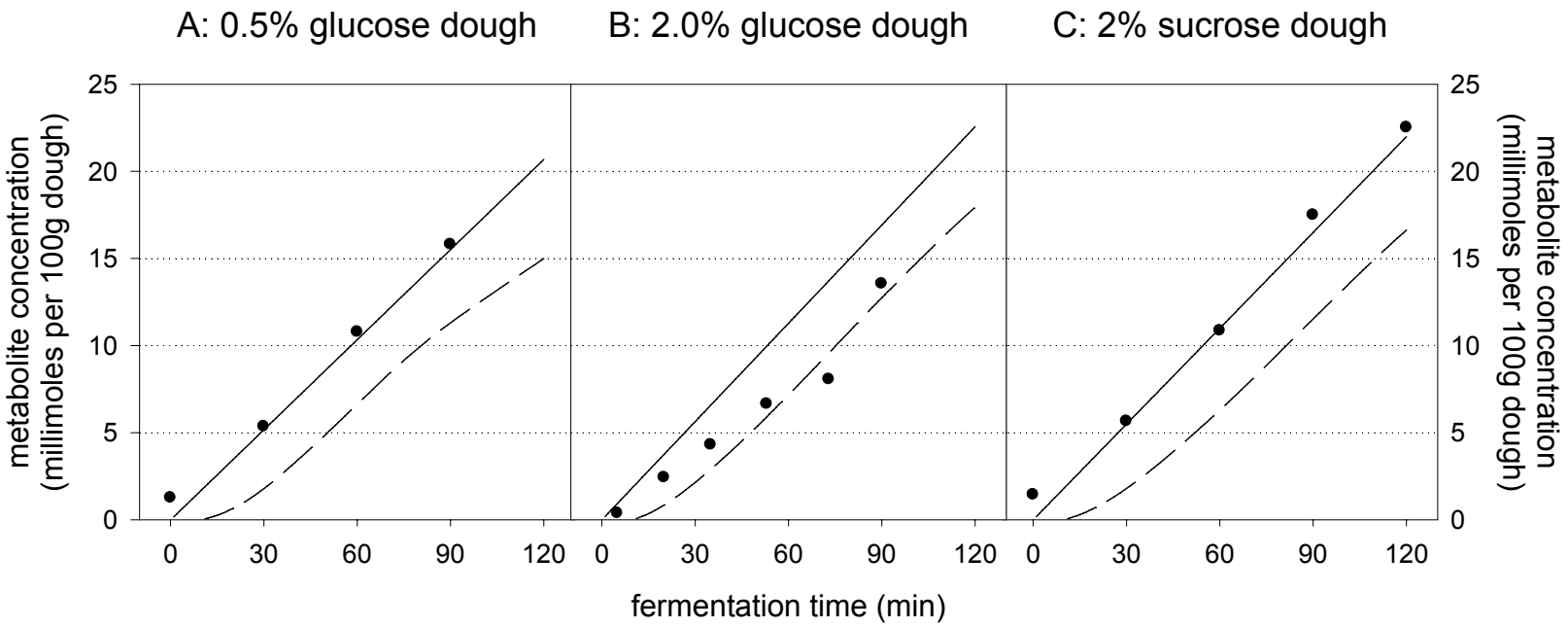
Upper and lower bounds are plotted below for five doughs in Figure 6.11, and ethanol is also shown. Where ethanol was added to dough, the added amount was subtracted from total measured ethanol.

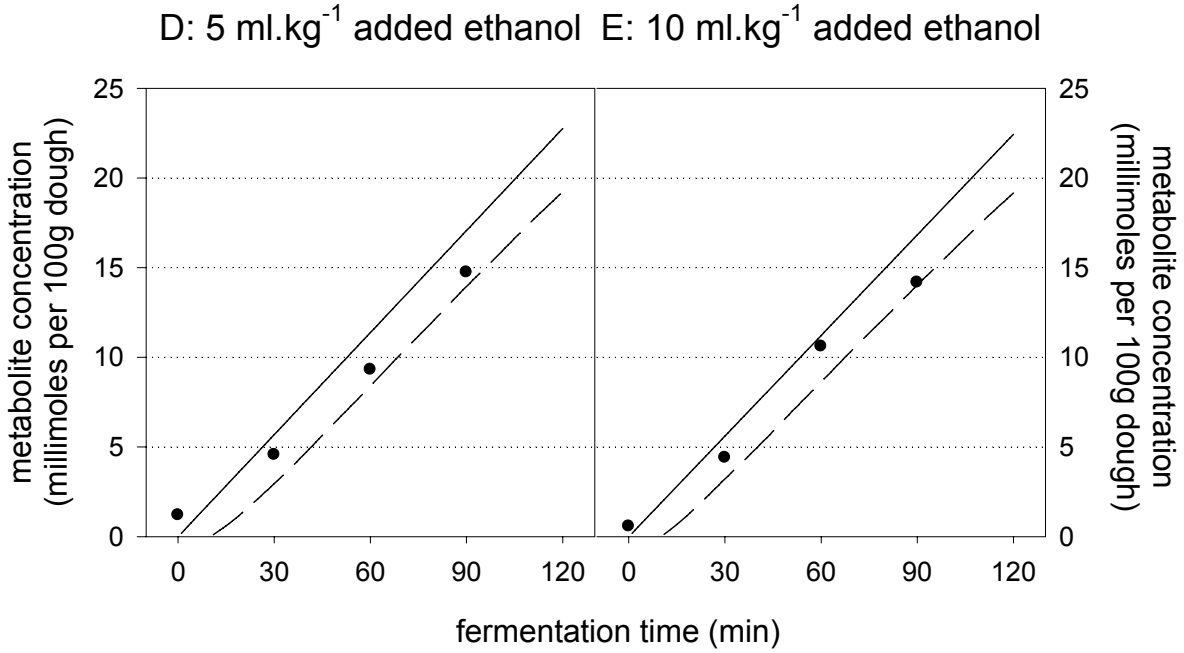
Figure 6.11. Ethanol-carbon dioxide mass balances for doughs fermenting at 30˚C.

Circles denote ethanol and solid and dashed lines denote upper and lower bounds of carbon dioxide production (respectively).

All doughs were made in a breadmaker with 600 g flour, 330ml water, 20 g yeast, 20 g oil, and 10 g salt plus extra ingredients: A 5g glucose, B 20g glucose, C 20g sucrose, D 20g sucrose and 5ml 95% ethanol, E 20g sucrose and 10ml 95% ethanol.

The upper bound was calculated by assuming carbon dioxide production at a constant rate equal to the maximum rate recorded in the Risograph. The lower bound is the Risograph cumulative gassing curve, averaged over two or three replicate determinations. Ethanol data are the average of duplicate assay results and added ethanol was subtracted from total measured ethanol.


A: 0.5% glucose dough
B: 2.0% glucose dough
C: 2% sucrose dough
metabolite concentration (millimoles per 100g dough)
metabolite concentration (millimoles per 100g dough)
0
5
10
15
20
25
0
30
60
90
120
fermentation time (min)


D: 5 ml.kg-1 added ethanol E: 10 ml.kg-1 added ethanol
metabolite concentration (millimoles per 100g dough)
metabolite concentration (millimoles per 100g dough)
0
5
10
15
20
25
0
30
60
90
120
fermentation time (min)

In doughs made with 0.5% glucose or 2% sucrose (Figure 6.11 A and C) ethanol was slightly above the upper bound because some was present at the start of fermentation. The 2.0% glucose dough produced less ethanol than other doughs, but ethanol mostly remained between upper and lower bounds.

In doughs with added ethanol (Figure 6.11 D and E) the gap between upper and lower bounds was smaller. This was because doughs with added ethanol attained maximal gassing rate faster than other doughs, and remained at that rate for longer (compare Figure 5.7 with Figure 5.3 and Figure 5.2). Reasons for this are unknown. Ethanol fell more evenly between the upper and lower bounds, and remained between them during 90 minutes of fermentation.

Reductive metabolism would theoretically yield equimolar amounts of carbon dioxide and ethanol, whereas oxidative metabolism would produce six moles of carbon dioxide per mole of hexose and no ethanol. The close correspondence seen here between molar quantities of ethanol and carbon dioxide arrived at by different methods suggests that they were accumulating in nearly equimolar amounts (Figure 6.11). Oxidative metabolism appears to be quantitatively unimportant.

6.10. Dissolved solutes in fermented dough

Each sugar molecule metabolised by anaerobic glycolysis yields two molecules each of ethanol and carbon dioxide – a net four-fold increase in the number of molecules. Fermentation would theoretically raise osmotic pressure in the aqueous phase and depress the initial freezing point. The effect of fermentation on initial freezing point was estimated using an equation for freezing point depression in ideal dilute solutions:

$$\Delta T_f = K_f m \qquad 6.6$$

ΔT_f	freezing point depression	[˚C]
K_f	cryoscopic constant, 1.86 for water (Lide 2004)	[˚C.mol^{-1}.kg]
m	molality of solution	[mol.kg^{-1}]

The aqueous phase of dough does not behave as an ideal dilute solution, but equation 6.6 gives a first approximation of freezing point depression. The effect of sugars, ethanol and salt on estimated freezing point of dough is shown in Table 6.8.

No information was found in the literature regarding changes in water mobility during fermentation and for calculation of molalities it was assumed that the amount of solvent water in dough remained constant.

Other solutes from flour, compressed yeast and water will also be dissolved in the aqueous phase, but these will not be affected by yeast metabolism. Carbon dioxide produced by yeast dissolves in the dough phase during fermentation. The mole fraction of CO_2 in water saturated with CO_2 at atmospheric pressure and 30˚C is 0.571 x 10^{-3} (Lide 2004), i.e.

$$\frac{n_{CO_2}}{n_{H_2O} + n_{CO_2}} = 0.571 \times 10^{-3} \qquad 6.7$$

Assuming that $n_{H2O} >> n_{CO2}$ and in one litre n_{H2O} = 55.5 mol, the concentration of dissolved CO_2 is 55.5 x 0.571 x 10^{-3} = 0.0317 mol.L^{-1}. The contribution of dissolved carbon dioxide to overall osmotic pressure would be small relative to that of other solutes.

According to Table 6.8 solute concentration is dominated by low molecular weight Na^+ and Cl^- ions, and the estimated change in freezing point due to 90 minutes of fermentation is only 0.7˚C. Attempts were made to measure the initial freezing point of dough by undercooling several degrees and seeding with an ice crystal. However it was difficult to induce freezing in undercooled dough and the equipment available was not sufficiently sensitive. When freezing was successfully induced, initial freezing points were between -3.1 and -4.2˚C in unyeasted dough with 2.0% added sucrose. Adding 10 ml.kg^{-1} 95% ethanol depressed the onset of freezing to -4.0 to -4.6˚C.

While the osmotic pressure wouldn't change markedly during fermentation, 6-carbon sugar molecules with five –OH groups would be replaced by 2-carbon ethanol molecules with one –OH group. There would be fewer –OH groups in solution and fewer water molecules would form hydrogen bonds with solutes. Small ethanol molecules would be able to penetrate into gluten proteins and interact with both polar residues (hydrogen bonding via the –OH group) and nonpolar residues (van der Waals interactions via the CH_3-CH_2 backbone).

Ethanol may influence the swelling of gluten proteins and the partition of lipids and other components between gluten and aqueous phases (Tolstoguzov 1997). However data from model systems indicate that the effect of ethanol on protein and lipid phase behaviour in dough is likely to be small (Eliasson and Larsson 1993).

Comment that ethanol depresses freezing point more than predicted from cryoscopic equation – see 'freezing model.xls'

Table 6.8. Solute concentrations and estimated freezing point depression in fermenting doughs made with 2% added sucrose.

	fermentation time (min)[A]	0	30	90
	solute	solute concentration mmol.(100g dough)$^{-1}$		
unheated	glucose	6.66	5.12	2.23
	fructose	9.55	9.51	7.02
	sucrose	0.8	1.68	1.15
	maltose	2.47	3.47	2.96
	ethanol	1.44	5.66	17.49
	Na^+ and Cl^- ions from salt[C]	34.19	34.19	34.19
	total	55.11	59.63	65.04
	aqueous phase concentration[D] (mol.kg^{-1})	2.14	2.32	2.53
	freezing point depression (˚C)[E]	3.99	4.32	4.71
	fermentation time (min)	0	30	90
	solute	solute concentration mmol.(100g dough)$^{-1}$		
heated[B]	glucose	6.46	4.74	1.64
	fructose	10.02	9.59	8.51
	sucrose	0.93	0.55	0.51
	maltose	3.18	3.31[F]	3.63[G]
	ethanol	1.44	5.66	17.49
	Na^+ and Cl^- ions from salt	34.19	34.19	34.19
	total	56.22	58.04	65.97
	aqueous phase concentration (mol.kg^{-1})	2.19	2.26	2.57
	freezing point depression (˚C)	4.07	4.20	4.77

A. doughs fermented at 30˚C.

B. extracts for sugar analysis heated at 85˚C for 1 h prior to assay.

C. 1% salt in dough formulation, assumed to completely dissociate into Na^+ and Cl^- ions

D. assuming 24.0 g solvent water per 100 g dough and neglecting other dissolved solutes

E. approximate freezing point depression calculated with the ideal cryoscopic equation

F. interpolated between 0 and 60 minutes

G. interpolated between 60 and 120 minutes

6.11. Summary and conclusions

A rapid, accurate method for extracting and analysing sugars and ethanol in dough was developed. Reproducibility was improved by heating extracts to denature enzymes.

Flour contained substantial amounts of sucrose and maltose but very little glucose or fructose. In unyeasted doughs maltose increased slowly during fermentation but other sugars changed little. The presence of yeast led to sucrose hydrolysis and liberation of fructose from an unknown source during mixing. Yeast cells consumed glucose in preference to fructose, and did not consume maltose during three hours of fermentation.

The molar yields of ethanol from glucose and fructose were 1.82 and 1.87 respectively. There was a close correspondence between the consumption of sugars and the accumulation of ethanol. The link between ethanol and carbon dioxide was more tenuous but appeared to confirm that oxidative metabolism was quantitatively unimportant.

The consequence of sugar metabolism in dough during fermentation is a relatively small change in osmotic pressure, which is dominated by low molecular weight solutes such as NaCl. Calculations that assumed ideal solution behaviour indicated little change in initial freezing point. However the assumptions behind these calculations are unlikely to be entirely applicable, and the results do not rule out solute-specific effects of ethanol.

7. YEAST METABOLITE DYNAMICS IN FROZEN DOUGH

7.1. Introduction

Preliminary experiments in this laboratory showed that post-thaw gassing power was adversely affected by a 90-minute pre-fermentation, and that the effect occurred following storage for 48 hours at -20˚C (section 1.5.3). Although published data on post-thaw gassing power were readily available, no previous studies had measured yeast metabolites other than carbon dioxide in frozen-thawed dough.

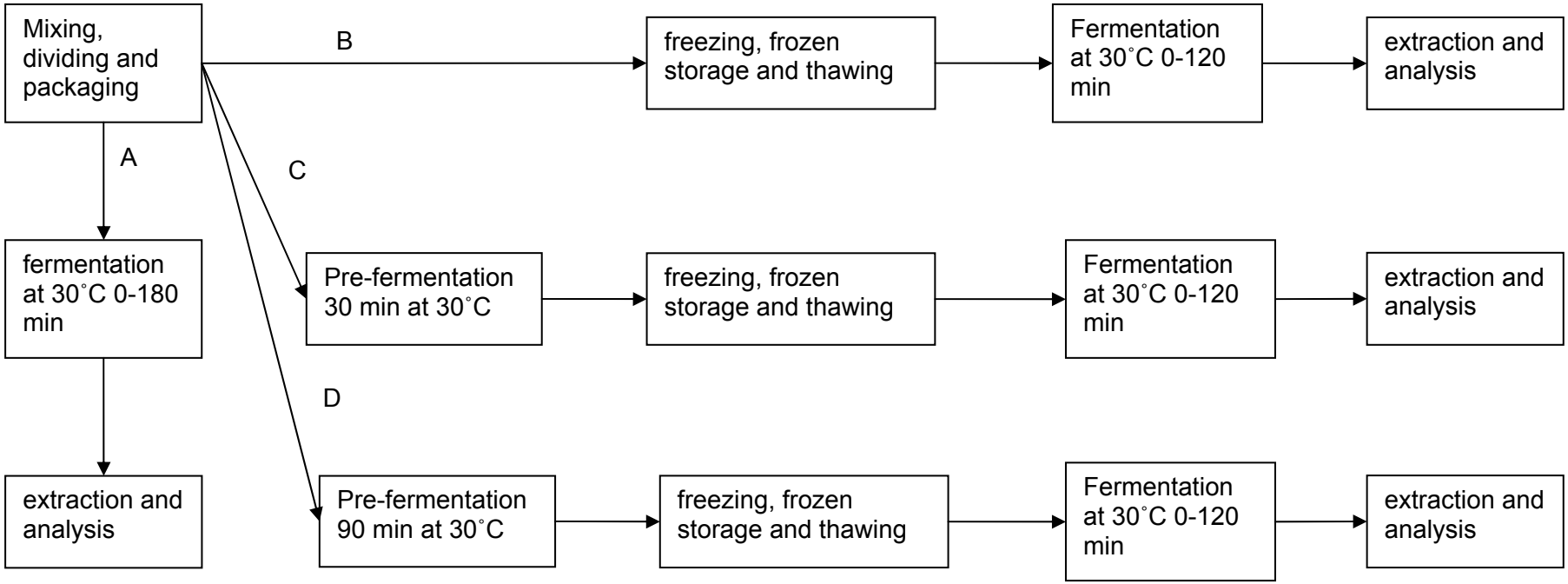
The experiments in this chapter were designed to quantitatively compare the consumption of sugars and excretion of metabolic byproducts so that comparisons with pre-freezing fermentation could be made.

An experiment was run to measure the changes in yeast metabolites during pre-fermentation and after freezing, frozen storage and thawing of unfermented or pre-fermented doughs. A 1.5kg dough made with 2% added sucrose was divided into 50 gram subsamples which were packaged in plastic bags and allocated into four streams, as shown in Figure 7.1:

A. Unfrozen dough
B. Unfermented frozen-thawed dough: frozen at -20˚C in a glycol bath immediately after mixing, stored 48 hours at -18˚C in a chest freezer and thawed at 0˚C for 1 h.
C. 30 minute pre-fermented dough: fermented 30 minutes at 30˚C then frozen at -20˚C in a glycol bath, stored 48 hours at -18˚C in a chest freezer and thawed at 0˚C for 1h.
D. 90 minute pre-fermented dough: fermented 90 minutes at 30˚C then frozen at -20˚C in a glycol bath, stored 48 hours at -18˚C in a chest freezer and thawed at 0˚C for 1h.

Following these treatments one or two samples of each dough were placed in Risograph cans and gassing power was measured. All other samples were fermented at 30˚C for up to 180 minutes. Samples were taken every 30 minutes and the sugar and ethanol contents measured. Dough extracts were not heated prior to extraction in this experiment. It is important to note that this experiment involved fermentation prior to freezing (0-90 minutes) as well as fermentation after thawing (0-180 minutes).

Figure 7.1. Outline of the experimental method for measuring yeast metabolites in unfermented and pre-fermented dough before and after freezing.



7.2. Gassing power

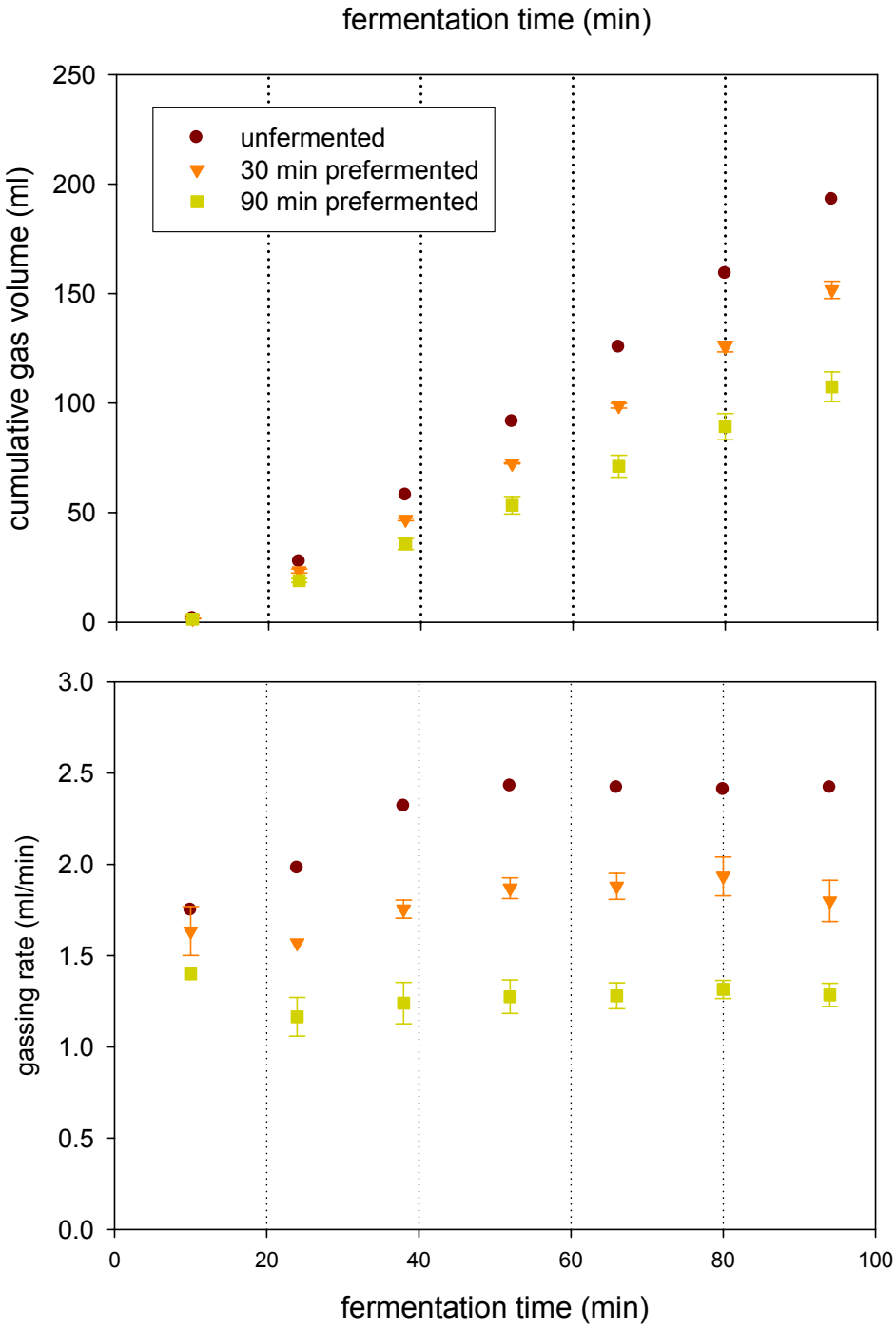
Gassing power of 30 minute pre-fermented doughs was lower than unfermented doughs, in contrast with other results from this laboratory (Shannon 2003) (compare Figure 7.2, next page, with Figure 1.4). This is probably because doughs in this experiment were pre-fermented at 30˚C, and fermentation would have been more rapid than in the earlier work, in which doughs were fermented at room temperature (approximately 20˚C).

In all doughs there was an initial dip in gassing rate, which took approximately 40 minutes to reach a constant level (Figure 7.2). This probably reflected warming of the doughs from the thawing temperature (0˚C) to the Risograph water bath temperature (30˚C).

As dough warms, the solubility of dissolved CO_2 falls and it evaporates from the aqueous phase into gas cells or surrounding air. Carbon dioxide evaporation and thermal expansion of gas cells would be registered by the Risograph as an increase in volume, which would artificially inflate gassing rate at first.

The increase in gassing rate subsequent to the initial dip could be explained by the effect of increasing temperature on yeast activity. The increase was small in 90 minute pre-fermented doughs, which may indicate that gas production was not temperature-limited.

Figure 7.2. Gas production by pre-fermented frozen-thawed doughs



A 1.5kg dough was made with 2% sucrose and divided into 50g subsamples. Dough samples were pre-fermented 0, 30 or 90 minutes at 30˚C, frozen and stored at -18˚C for 48 h then thawed at 0˚C for 1 h and transferred to Risograph cans.

Data collected during the first ten minutes were discarded to allow for thermal expansion of air in the Risograph can. Gassing power measurements on the unfermented control dough were not replicated. The gassing power of pre-fermented doughs was measured in duplicate and vertical bars are standard deviations.

7.3. Sugars and ethanol

There were no significant differences between glucose and fructose levels immediately before freezing and after thawing (Table 7.1), which indicated that yeast metabolism during freezing, frozen storage and thawing was minimal.

Table 7.1. Glucose and fructose concentrations in dough before freezing and immediately after thawing.

pre-fermentation time (min)	sugar	mmol.(100g dough)$^{-1}$		statistical significance
		pre-freezing	post-thawing	
0	glucose	6.31 ± 0.38[A]	5.78 ± 0.25	ns[B]
	fructose	8.79 ± 1.41	9.36 ± 1.09	ns
30	glucose	4.09 ± 0.48	3.51 ± 0.10	ns
	fructose	10.03 ± 1.49	9.22 ± 0.47	ns
90	glucose	1.18 ± 0.16	1.11 ± 0.11	ns
	fructose	5.43 ± 0.53	5.11 ± 0.47	ns

A. mean ± standard deviation of results from two extractions analysed in duplicate

B. difference between pre-freezing and post-thawing sugars was not significant at 95% confidence

Figure 7.3. Sugars and ethanol in unfermented and pre-fermented dough before and after freezing.

A 1.5kg batch of dough was divided into 50 g subsamples, which were allocated into four streams: stream A was fermented at 30˚C, stream B was immediately frozen, stream C was fermented for 30 minutes at 30˚C then frozen and stream D was fermented at 90 minutes at 30˚C then frozen.

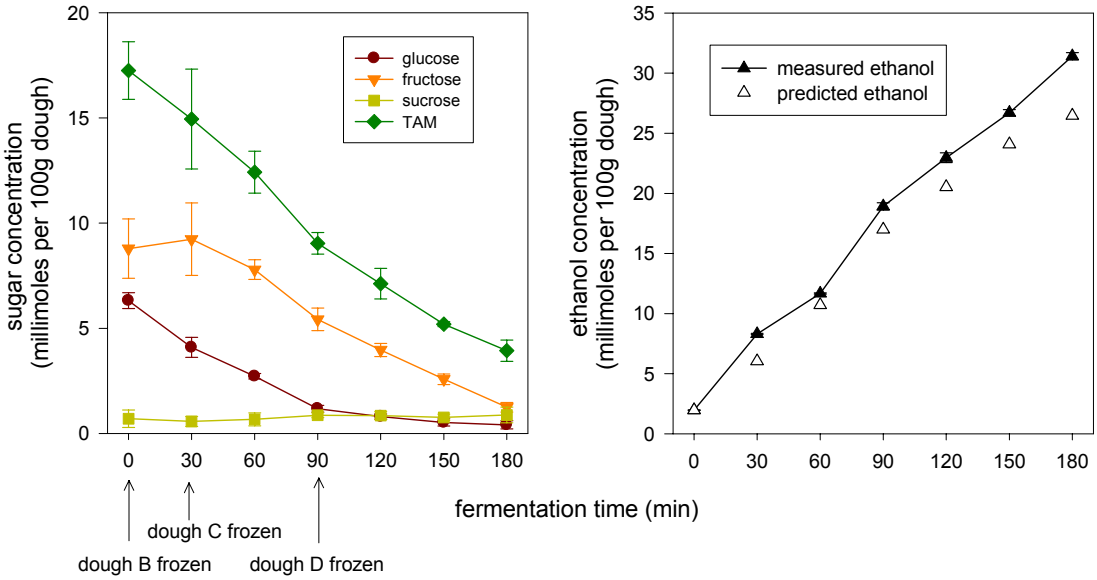
Samples of the unfrozen dough (stream A) were analysed for glucose, fructose, sucrose and ethanol at various times during 180 minutes of fermentation. Frozen doughs were stored at -18˚C for 48 h then thawed at 0˚C for 1 h and fermented at 30˚C. Samples were extracted at various times during 120 minutes of post-thaw fermentation and extracts were assayed for sugars and ethanol. All samples were extracted without heating.

TAM (total available monosaccharides) was calculated as (moles glucose) + (moles fructose) + 2 x (moles sucrose). Ethanol was measured in fermenting doughs or predicted from the consumption of glucose, fructose and sucrose using experimentally-determined molar yields.

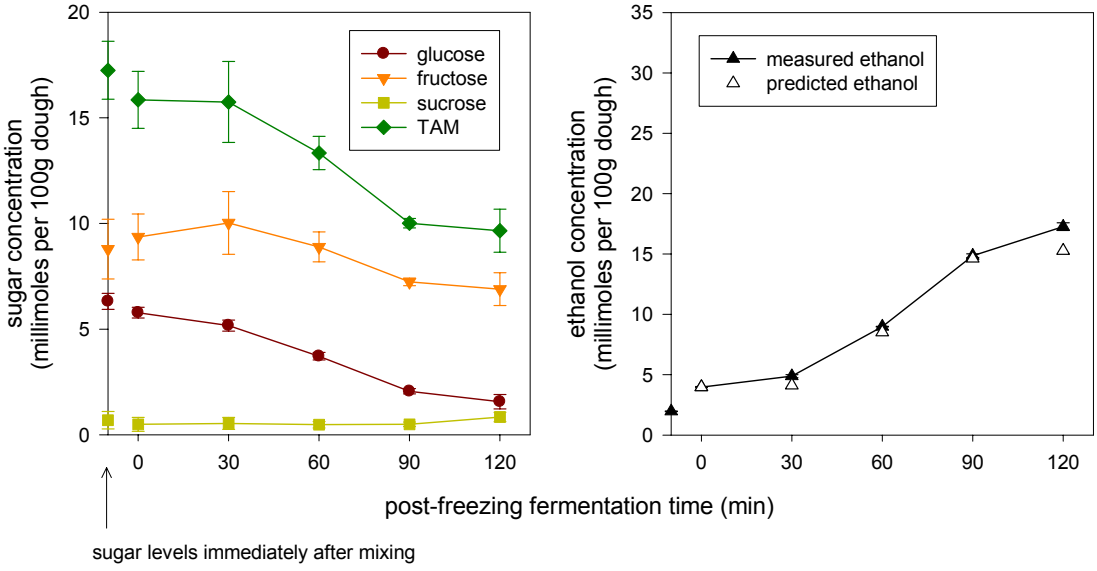
Points on the y-axis for frozen doughs indicate metabolite levels immediately before the start of freezing.

Ethanol data are the mean of duplicate assays and sugar data are the means of duplicate assays on duplicate extracts. Vertical bars are standard deviations.

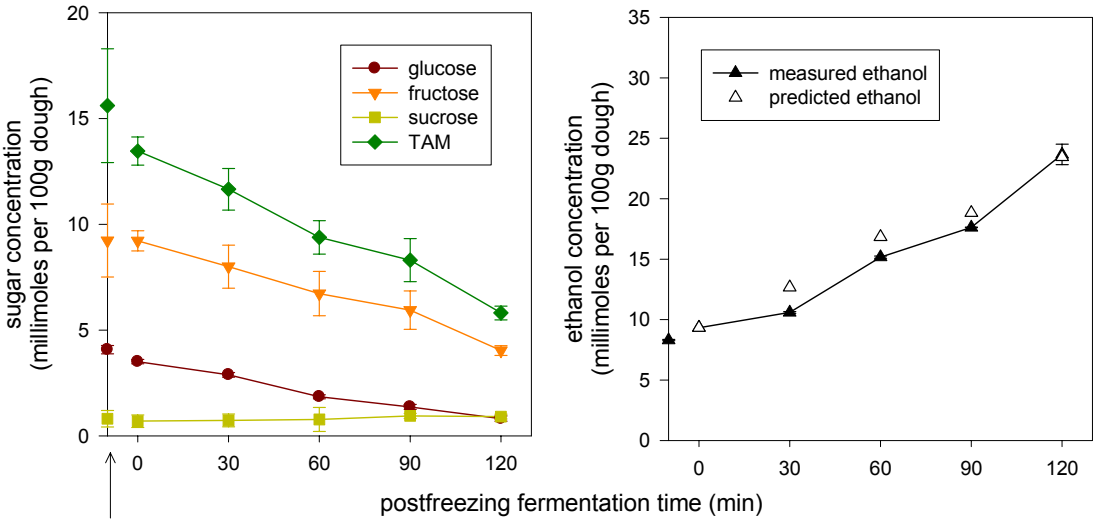
A: Unfrozen dough



B: Unfermented frozen-thawed dough

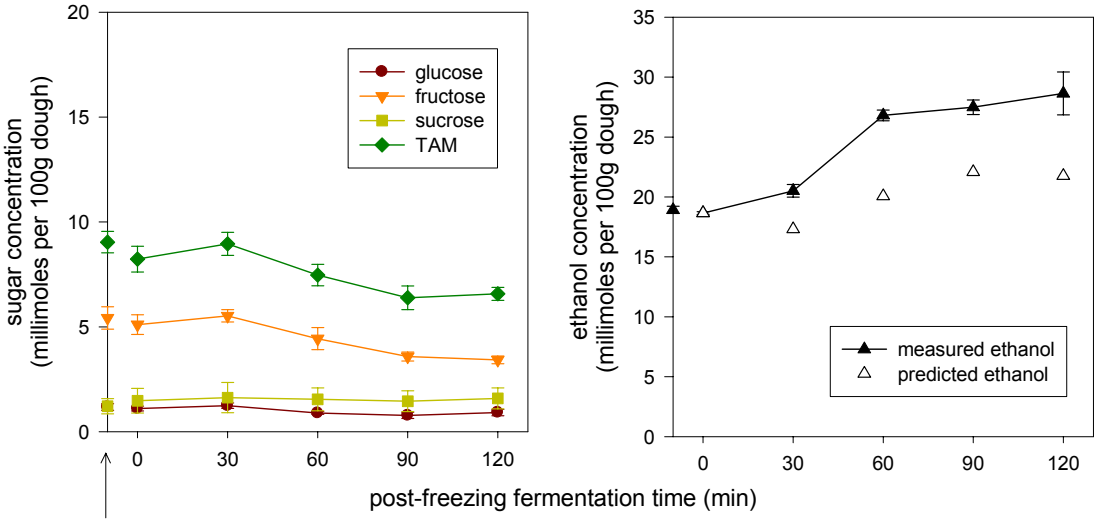


C: 30 min prefermented frozen-thawed dough



sugar levels after 30 minutes prefermentation

D: 90 min prefermented frozen-thawed dough



sugar levels after 90 minutes prefermentation

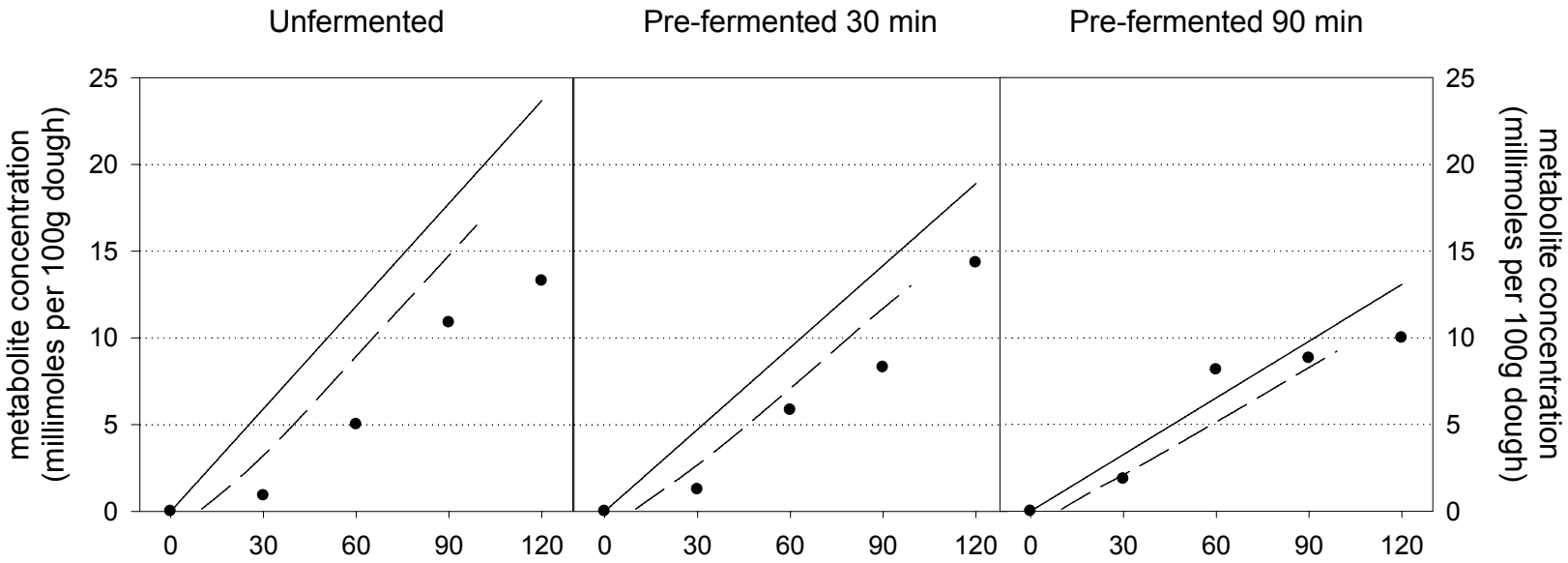
In all doughs glucose was consumed faster than fructose. In frozen-thawed doughs sugar consumption and ethanol production were slow during the first 30 minutes after thawing. This is probably because the doughs were initially at 0˚C and took almost an hour to equilibrate to 30˚C in the temperature-controlled room (Appendix A4).

There was a good correspondence between predicted and measured ethanol in unfrozen dough, unfermented frozen-thawed dough and 30 minute pre-fermented frozen-thawed dough. Ethanol production was apparently less sensitive to a 30 minute pre-fermentation than gas production.

In 90 minute pre-fermented dough ethanol exceeded predictions based on the decline in glucose, sucrose and fructose. This may indicate that yeast consumed an alternative sugar source but a more likely explanation is that the discrepancy was an artifact of the extraction procedure.

Doughs pre-fermented 90 minutes contained almost 20 mmol.(100g dough)$^{-1}$ ethanol but produced ethanol at a rate comparable to other doughs during the first hour of post-thaw fermentation. This is in agreement with the findings that 16.9 mmol.(100g dough)$^{-1}$ added ethanol did not inhibit fermentation in unfrozen doughs.

Figure 7.4. Ethanol-carbon dioxide mass balances in pre-fermented frozen-thawed doughs.



A 1.5kg dough was made with 2% sucrose and divided into 50g subsamples. Dough samples were pre-fermented 0, 30 or 90 minutes at 30˚C, frozen and stored at -18˚C for 48 h then thawed at 0˚C for 1 h. Samples were transferred to Risograph cans or fermented at 30˚C for various times before extraction for ethanol measurement.

Circles denote ethanol, and upper and lower bounds of carbon dioxide production are solid and dashed lines respectively. The upper bound was calculated by assuming carbon dioxide production at a constant rate equal to the maximum rate recorded in the Risograph. The lower bound is the Risograph cumulative gassing curve. Ethanol data are the mean of duplicate assay results minus the ethanol present at the completion of thawing.

For unfermented and 30 minute pre-fermented doughs the Risograph recorded gas production in excess of the ethanol recovered (Figure 7.4). This may be due to evaporation of dissolved CO_2 as doughs warmed from 0˚C to 30˚C.

The mole fraction of carbon dioxide in a saturated aqueous solution at 100 kPa is 0.571 x 10^{-3} at 30˚C and 1.337 x 10^{-3} at 0˚C (Lide 2004), i.e. an increase of 134% with 30˚C cooling. Hibberd and Parker (1976) calculated that their doughs contained 0.81 ml.(g dough)$^{-1}$ dissolved CO_2 at 27˚C. A 134% increase would give 1.90 ml.g^{-1} dissolved at 0˚C, i.e. 1.09 ml.g^{-1} more CO_2 would be dissolved at 0˚C than at 30˚C. Converting to molar units using the molar volume from equation 6.5 gives 4.41 mmol.(100g dough)$^{-1}$.

If 4.41 mmol.(100g dough)$^{-1}$ of gas production over the first hour was attributed to evaporation of CO_2 already dissolved in the dough at the start of fermentation, the remainder would coincide quite well with ethanol in unfermented and 30 minute pre-fermented doughs (Figure 7.4). This effect appeared to be absent in the 90 minute pre-fermented doughs, in which ethanol and CO_2 were almost the same. The reason for this is not known.

7.4. Summary and conclusions

Pre-fermentation of 30 minutes or longer caused a reduction in gassing power after freezing, 48 hours' frozen storage and thawing. Sugar was not consumed during freezing, storage or thawing. The stoichiometric ratios between consumption of glucose or fructose and production of ethanol during pre-fermentation were preserved after thawing, with the exception of the 90-minute pre-fermented dough. Replication was insufficient to judge the significance of this departure.

The correspondence between carbon dioxide and ethanol in fermenting frozen-thawed doughs was not as close as in pre-fermenting dough. This could be partly explained by the effect of dough temperature on carbon dioxide dissolution equilibria.

8. MATHEMATICAL MODELLING

8.1. Introduction

In the preceding sections the average composition of doughs with respect to yeast metabolites and metabolic byproducts was determined during pre- and post-freezing fermentation. Indications from this work and the literature were that dough composition changed during pre-fermentation in such a way that freezing, frozen storage and thawing injured yeast cells or otherwise prevented them from fermenting normally after thawing. The mathematical models in this section examine the spatial distribution of ethanol around fermenting yeast cells (micro model) and provide a tool to predict the amount of sugars and ethanol in dough at any time during 3 hours of fermentation.

There have been few systematic studies of sugar uptake in dough, and none have applied kinetic principles common in other fermented food industries (Wang et al. 2004, Kurz et al. 2002) and enzymology (Cornish-Bowden 1995; Smogrovicova et al. 2001).

There are contrasting reports regarding the rate of yeast cell division in fermenting dough (Hoffman et al. 1941; Thorn and Ross 1960; Akdogan and Ozilgen 1992; Vivier et al. 1994). Reliable methods for enumerating yeast cells *in situ* have been reported (Autio and Mattila-Sandholm 1992), but they have yet to be systematically applied to bakery research.

Findings in section 6.9 suggested that yeast cells were metabolising reductively after mixing, dividing and packaging, which took up to 28 minutes (section). The ATP yield from reductive metabolism is low, and as a consequence growth rates are lower and sugar uptake more rapid than during respirofermentative growth. Biomass yield from reductive metabolism is low, and it was assumed here that cell division in fermenting dough was negligible.

8.2. Microsystem model

8.2.1 Model development

It has been suggested that the accumulation of volatile fermentation wastes in dough may be partly responsible for the deleterious effect of pre-fermentation on post-thaw gassing power (Tanaka and Miyatake 1975; Tanaka et al. 1976; Hsu et al. 1979a). The effect of pre-fermentation on post-thaw gassing power is partly reproduced by adding fermentation wastes or ethanol to dough (Tanaka and Miyatake 1975; Tanaka et al. 1976), but the question of whether this is equivalent to *in situ* accumulation of fermentation metabolites has not been addressed.

If ethanol diffused away from a cell in dough more slowly than it was excreted, it would build up in the vicinity of the cell. The bulk concentration of metabolites would not be representative of the conditions experienced by the yeast cell. Water crystallisation during freezing would boost the ethanol concentration around a yeast cell to potentially damaging levels.

A related issue is that if sugar diffused towards the cell more slowly than it was consumed, consumption of sugar could theoretically create a gradient of sugar concentration around cells. If sugar consumption was diffusion-limited, a deficit of sugar immediately adjacent to the cell could inhibit fermentation and gas production.

Any gradients of ethanol or sugar that persisted in the dough during freezing would drive diffusion during frozen storage, and such phenomena could be linked with the rapid loss of shelf life in pre-fermented frozen dough.

It is therefore pertinent to ask whether consumption of sugar and excretion of waste products occur on the same time-scale as their diffusion through the aqueous phase. This was addressed with a mathematical model.

The model was conceived as a spherical yeast cell inside a sphere of dough (Figure 8.1). A line was drawn from the centre of the yeast cell to the surface of the sphere (Figure 8.2), passing through the yeast cell (yeast zone) and dough around the cell (dough zone). The dough zone could contain aqueous phase, solid material or a mixture of both. The case in which only aqueous phase is encountered was modelled here.

Figure 8.1. Schematic of a yeast cell excreting ethanol and consuming sugar in a surrounding spherical region of dough.

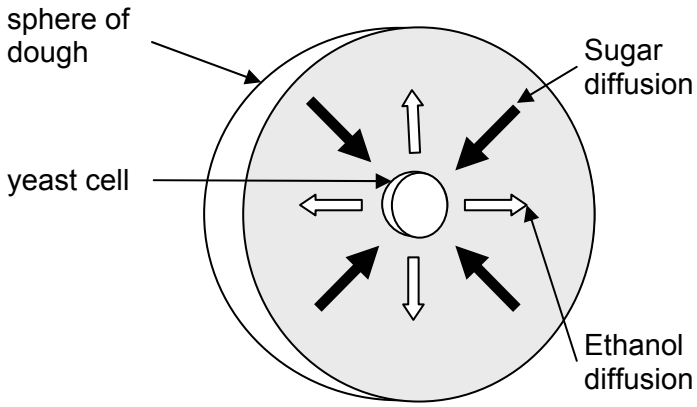
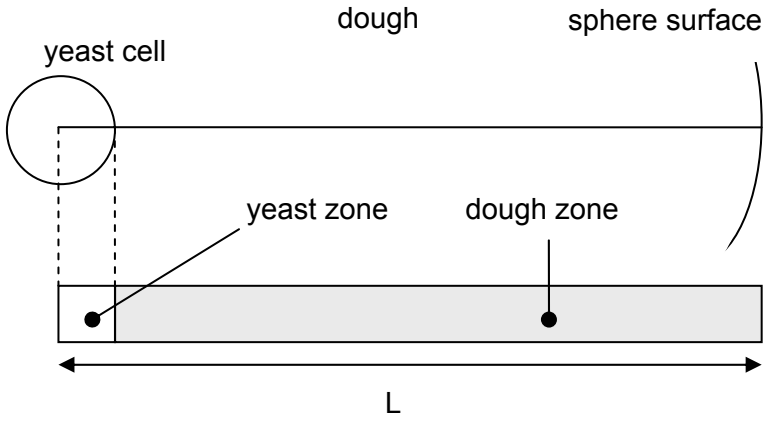


Figure 8.2. Schematic showing a line drawn from the centre of a yeast cell to the surface of a sphere of dough surrounding it.



The microsystem model described glucose and ethanol concentrations at points along the line in Figure 8.2. Yeast metabolism was simulated by decreasing the sugar concentration in the yeast zone at each time step, which created a concentration gradient. Diffusion to resolve the gradient was simulated with the diffusion equation.

Section 6 showed that glucose and fructose were consumed in preference to other sugars in fermenting dough, and that yields of ethanol from the two were similar. The microsystem model considers only glucose, but the diffusion coefficient of fructose is close to that of glucose (Wong 1997; Lide 2004) and it is therefore reasonable to expect similar diffusion behaviour.

8.2.2 Parameters

The hypothetical sphere of dough in Figure 8.1 represents the average volume of dough per yeast cell. The average volume per cell was calculated as the inverse of the average number of cells per unit volume of dough.

Autio and Mattila-Sandholm (1992) stained 10 µm slices of dough with a fluorescent stain that identified actively-metabolising yeast cells. The average density of active cells in dough made with 1% compressed yeast was 320 per square millimeter. A more realistic yeast level for frozen dough is 2%, which would theoretically give an average density of 2 x 320 = 640 mm^{-2}. The average area per yeast cell would then be 640^{-1} mm^2.

For simplicity, the area of the dough allocated to each yeast cell was visualized as a circle with area $640^{-1}mm^2$. The radius of the circle was then calculated from its area:

$$r_{dough} = \sqrt{\frac{A_{dough}}{\pi}} = \sqrt{\frac{640^{-1}}{\pi}} = 0.0223mm \qquad 8.1$$

r_{dough} radius of the hypothetical circle of dough allocated to each yeast cell [mm]

A_{dough} area of dough per yeast cell (inverse of 2-dimensional density) [mm^2]

If yeast cells were homogeneously distributed in dough, a slice in any direction would give the same cell density. The hypothetical circle could therefore be expanded to a sphere with the same radius, which is the radius of the sphere in Figure 8.1 and the length of the radial line in Figure 8.2, i.e. $L = r_{dough}$.

Saccharomyces cerevisiae cells are typically ovoid, and their longest dimension reaches approximately 6 µm (Robinow and Johnson 1991; Walker 1998). The size of the yeast zone in Figure 8.2, which represents half the diameter, was set at 3 µm. The diameter of the hypothetical sphere of dough allocated to each yeast cell is 7.4 times the width of the cell.

The aqueous phase water content (24.0 millilitres per 100 grams of dough) was used to calculate initial glucose concentration (G_i) in a dough containing 2% w/w glucose:

$$G_i = \frac{2\ g\ glucose}{100\ g\ dough} \cdot \frac{1\ mol}{180\ g} \cdot \frac{100\ g\ dough}{0.0240\ L\ solvent\ \ water} \qquad 8.2$$

$$= 0.432 \frac{mol}{L}$$

Initial ethanol concentration, E_i was set to zero.

The rate of glucose consumption, G_R, was calculated in section 6.7 from the rate at which glucose declined in fermenting dough (0.0071 mmol.(100g dough)$^{-1}$.min^{-1}). This was similarly converted to volumetric units:

$$G_R = \frac{0.0071 \times 10^{-3}\ mol\ glucose}{100\ g\ dough \cdot min} \cdot \frac{100\ g\ dough}{0.0257\ L\ solvent\ \ water} \qquad 8.3$$

$$= 0.00276 \frac{mol}{L \cdot min}$$

Diffusion coefficients for yeast metabolites in aqueous phase were not available in the literature. The nearest alternatives were for diffusion in binary aqueous solutions at infinite dilution – 0.67 x 10^{-5} cm^2.s^{-1} for glucose in water and 1.24 x 10^{-5} cm^2.s^{-1} for ethanol in water (Lide 2004). These were converted to units of m^2.s^{-1} by multiplying by 10^{-4}.

Diffusion coefficients are affected by viscosity according to the Stokes-Einstein equation (Atkins and De Paula 2002):

$$D = \frac{kT}{6\pi\eta a} \qquad 8.4$$

D	diffusion coefficient	[m^2.s^{-1}]
k	Boltzmann constant	[N.m.K^{-1}]
T	absolute temperature	[K]
η	coefficient of viscosity	[N.s.m^{-2}]
a	hydrodynamic radius of the diffusing species	[m]
π	3.14159	-

Mauritzen and Stewart (1965) reported that the viscosity of the supernatant separated from dough by ultracentrifugation was 250 centipoise, which is approximately 250 times the viscosity of pure water at 20˚C (James and Lord 1992). The diffusion coefficient is inversely related to viscosity (equation 8.4) and coefficients at infinite dilution were divided by 250 accordingly. Units were converted from $cm^2.s^{-1}$ to $m^2.s^{-1}$ by multiplying with a factor of 10^{-4}

Values of the simulation parameters are summarised in Table 8.1.

Table 8.1. Microsystem model parameters

symbol	*description*	*value*	*units*
L	length of the modelling domain	0.0223	mm
G_R	glucose consumption rate	0.00276	$mol.L^{-1}.min^{-1}$
G_i	initial glucose concentration	0.432	$mol.L^{-1}$
E_i	initial ethanol concentration	0	$mol.L^{-1}$
D_g	estimated diffusion coefficient for glucose	$2.68x10^{-12}$	$m^{2.}.s^{-1}$
D_e	estimated diffusion coefficient for ethanol	4.96×10^{-12}	$m^2.s^{-1}$
Y_{eg}	molar yield of ethanol from glucose	1.82	–

8.2.3 Mathematical treatment

Ideal diffusion of an uncharged species is described by the diffusion equation, sometimes known as Fick's second law (Saff and Nagle 1996; Atkins and De Paula 2002).

Fick's first law states that the number of particles passing through a unit area of space in unit time (the flux, *J*) is proportional to rate of change of concentration with distance. The constant of proportionality is the diffusion constant, *D*, and a negative sign is required because molecules diffuse from high to low concentration (equation 8.5).

$$J = -D\frac{\Delta c}{\Delta x} \qquad 8.5$$

J	diffusion flux	[mol.m^{-2}.s^{-1}]
D	diffusion coefficient	[m^2.s^{-1}]
c	solute concentration	[mol.m^{-3}]
x	distance	[m]

The limit of 8.5 as Δx becomes infinitesimally small is:

$$J = -D\frac{dc}{dx} \qquad 8.6$$

Figure 8.3 shows a region of space bounded by x and $x + \Delta x$ with cross-sectional area A. Molecules diffuse into this region from the high-concentration side and out towards the low-concentration side.

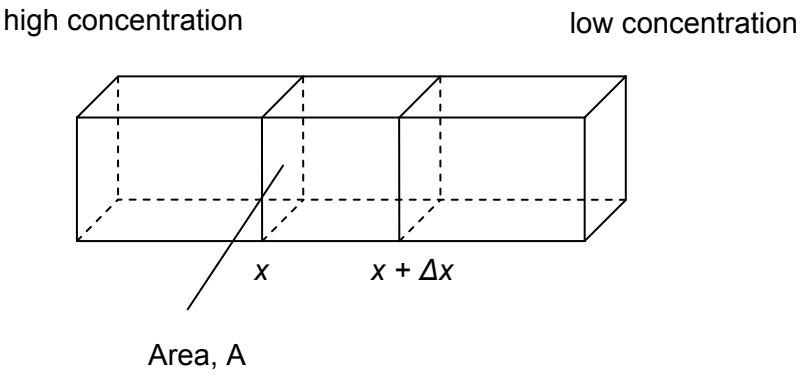


Figure 8.3. Schematic representation of a volume of space through which molecules diffuse down a concentration gradient from left to right.

The net change in the number of particles in the bounded region, Δn, is the difference between flux at x and flux at $x + \Delta x$:

$$\Delta n = J(x)A\Delta t - J(x + \Delta x)A\Delta t = (J(x) - J(x + \Delta x))A\Delta t \qquad 8.7$$

Δn	change in the number of particles in the region between x and $x + \Delta x$ during time period Δt	[mol]
$J(x)$	diffusion flux at position x in Figure 8.3	[mol.m^{-2}.s^{-1}]
A	cross-sectional area of the region bounded by x and $x + \Delta x$	[m^2]
Δt	time interval over which diffusion occurs	[s]

The flux at $x + \Delta x$ can be expressed in terms of the flux at x and the gradient of the flux:

$$J(x + \Delta x) = J(x) + \frac{\Delta J}{\Delta x} \times \Delta x \qquad 8.8$$

Substituting into equation 8.7 and dividing both sides by Δx and $A\,\Delta t$:

$$\Delta n = \left(J(x) - \left(J(x) + \frac{\Delta J}{\Delta x} \times \Delta x \right) \right) A \Delta t \qquad 8.9$$

$$\Delta n = -\frac{\Delta J}{\Delta x} \times \Delta x \times A \Delta t \qquad 8.10$$

$$\frac{\left(\frac{\Delta n}{A \Delta x} \right)}{\Delta t} = -\frac{\Delta J}{\Delta x} \qquad 8.11$$

The number of molecules divided by volume is concentration:

$$\frac{\Delta n}{A \Delta x} = \Delta c \qquad 8.12$$

Substituting 8.5 and 8.12 into 8.11:

$$\frac{\Delta c}{\Delta t} = -\frac{\Delta\left(-D \frac{\Delta c}{\Delta x} \right)}{\Delta x} \qquad 8.13$$

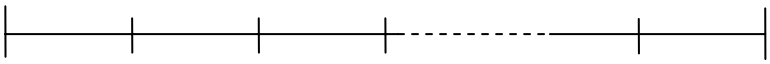
$$\frac{\Delta c}{\Delta t} = D \frac{\Delta\left(\frac{\Delta c}{\Delta x} \right)}{\Delta x} \qquad 8.14$$

This expression becomes more exact as Δt and Δx become smaller. Concentration is a function of both location and time, so the limit as time and space intervals become infinitesimal uses partial derivatives:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \qquad 8.15$$

8.2.4 Numerical solution

The line from the centre of the yeast cell to the surface of the hypothetical sphere of dough was partitioned into n intervals of width Δx. Partition points were labeled x_0, x_1, x_2 … x_n:



Partition point:

x_0 x_1 x_2 x_{n-1} x_n

Distance from cell centre:

0 Δx $2\Delta x$ $(n-1)\Delta x$ $n\Delta x = L$

Three hours of fermentation was simulated. The total time was divided into m intervals of size Δt seconds separated by points t_0, t_1, t_2 … t_m. Concentration at space point x_i and time point t_j is denoted c_{ij}.

At each time step the glucose concentration for partition points in the yeast cell zone was decreased to simulate consumption. The decrease was sufficient to lower average concentration over the modelling domain by an amount corresponding to the average rate of consumption seen in experimental results. For example, if L = 15 µm then concentration in the 3µm yeast cell zone would be decreased by five times the average rate. Ethanol concentration in the yeast zone was increased by an amount corresponding to the drop in glucose multiplied by the experimental yield of ethanol from glucose (section 6.7).

After glucose and ethanol were adjusted at points in the yeast zone, the resulting diffusion was predicted by numerically solving the one-dimensional diffusion equation (8.15). The left hand side of the diffusion equation was approximated with a forward difference formula:

$$\frac{\partial c}{\partial t} \approx \frac{1}{\Delta t}\left[c_{i,j+1} - c_{i,j}\right] \qquad 8.16$$

c	solute concentration	[mol.m^{-3}]
i	space partition point number	-
j	time partition point number	-

t time [s]

Δt time interval for numerical solution steps [s]

Using the implicit Crank-Nicolson method (Fausett 1999) the right hand side of 8.15 was approximated with the average of the centred difference at the forward time step t_{j+1} and the centred difference at the current time step t_j.

$$\frac{\partial^2 c}{\partial x^2} \approx \frac{D}{2}\left\{\frac{1}{\Delta x^2}\left[c_{i-1,j} - 2c_{i,j} + c_{i+1,j}\right] + \frac{1}{\Delta x^2}\left[c_{i-1,j+1} - 2c_{i,j+1} + c_{i+1,j+1}\right]\right\} \quad 8.17$$

Equating 8.16 with 8.17, grouping constants into a new constant R, and rearranging:

$$\frac{1}{\Delta t}\left[c_{i,j+1} - c_{ij}\right] =$$

$$\frac{D}{2}\left\{\frac{1}{\Delta x^2}\left[c_{i-1,j} - 2c_{i,j} + c_{i+1,j}\right] + \frac{1}{\Delta x^2}\left[c_{i-1,j+1} - 2c_{i,j+1} + c_{i+1,j+1}\right]\right\} \quad 8.18$$

$$R = \frac{D\Delta t}{\Delta x^2} \quad 8.19$$

$$c_{i,j+1} - \frac{R}{2}\left[c_{i-1,j+1} - 2c_{i,j+1} + c_{i+1,j+1}\right]$$

$$= c_{i,j} + \frac{R}{2}\left[c_{i-1,j} - 2c_{i,j} + c_{i+1,j}\right] \quad 8.20$$

$$-\frac{R}{2}c_{i-1,j+1} + (1+R)c_{i,j+1} - \frac{R}{2}c_{i+1,j+1}$$

$$= \frac{R}{2}c_{i-1,j} + (1-R)c_{i,j} + \frac{R}{2}c_{i+1,j} \quad 8.21$$

It was assumed that there was no diffusion across the boundaries of the modelling domain, i.e. that $dc/dx = 0$ at the boundary points x_0 and x_n. This is known as the

Neumann boundary condition. It is accommodated by adding a fictitious point to the grid at each end and setting concentration at the fictitious point equal to concentration at one point in from the boundary, i.e. $c_{-1,j} = c_{1,j}$ and $c_{n+1,j} = c_{n-1,j}$ (Fausett 1999).

Equation 8.21 can be expressed in matrix-vector form as **A**x = b where A is a tri-diagonal matrix of coefficients, x is a vector of unknown *c* values at time *j+1* and b is a vector containing the numerical values of the right hand side of 8.21. This is then solved for x at each time step using a matrix factorisation algorithm (Fausett 1999).

In summary, the diffusion equation and initial conditions are:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \qquad 8.22$$

$$0 < x < L \qquad 8.23$$

$$t, c > 0 \qquad 8.24$$

$$\frac{\partial c}{\partial t}(0,t) = \frac{\partial c}{\partial t}(L,t) = 0 \qquad 8.25$$

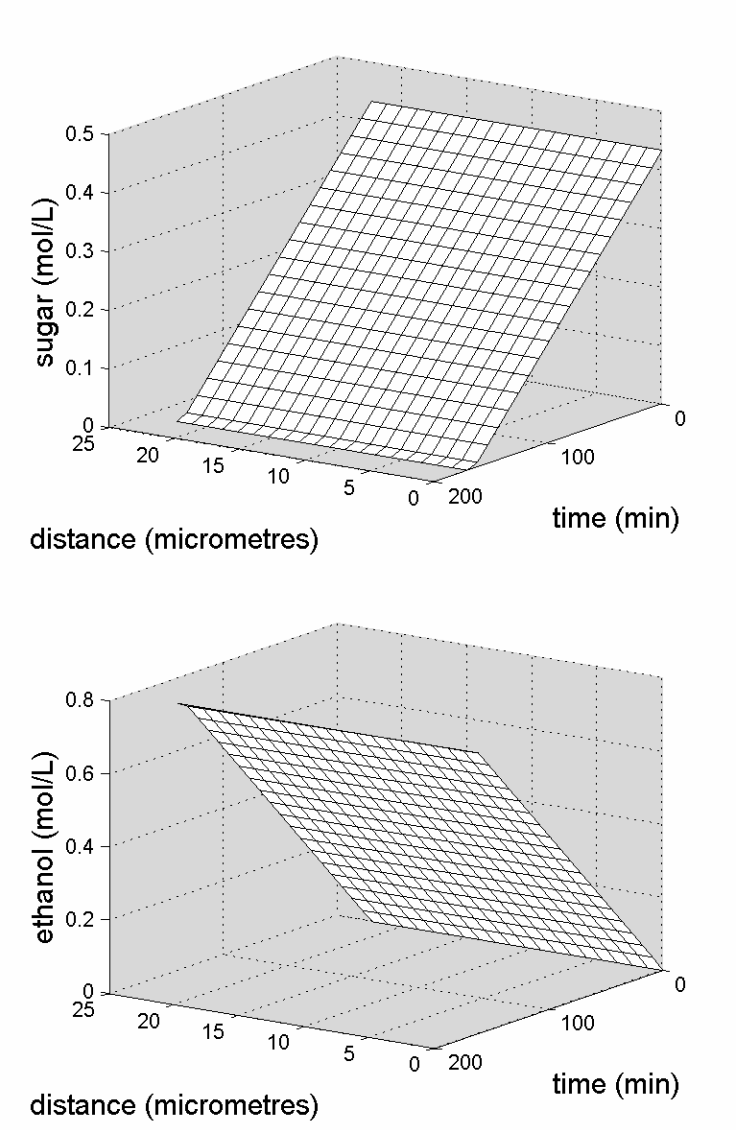
$$c(x,0) = c_{initial} \qquad 8.26$$

A program to evaluate the numerical solution was written in Matlab version 6.5 release 13 (The Mathworks Inc.). Program code is shown in Appendix A6. The simulation was run with 50 space points and time steps of 2 seconds.

8.2.5 Simulation results

The simulation was run with parameters in Table 8.1 and time scale 180 minutes. Each time step produced a vector of sugar and ethanol concentrations at points x_0 to x_n. Vectors are plotted along the 'distance' axis in Figure 8.4, which denotes the distance from the centre of the yeast cell. For clarity plots show a subset of data sampled at regular intervals from the whole dataset.

Figure 8.4. Simulated sugar (top) and ethanol (bottom) concentration around a yeast cell in fermenting dough.



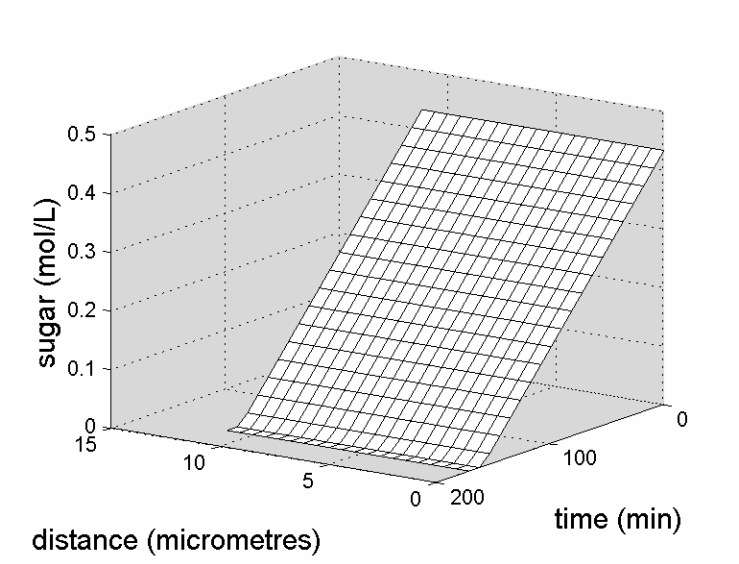
Concentrations were modelled in one dimension as a function of radial distance from the cell. The distance axis origin is at the centre of the yeast cell and lines parallel to the distance axis show concentration along a line radial to the cell, which passes through dough aqueous phase.

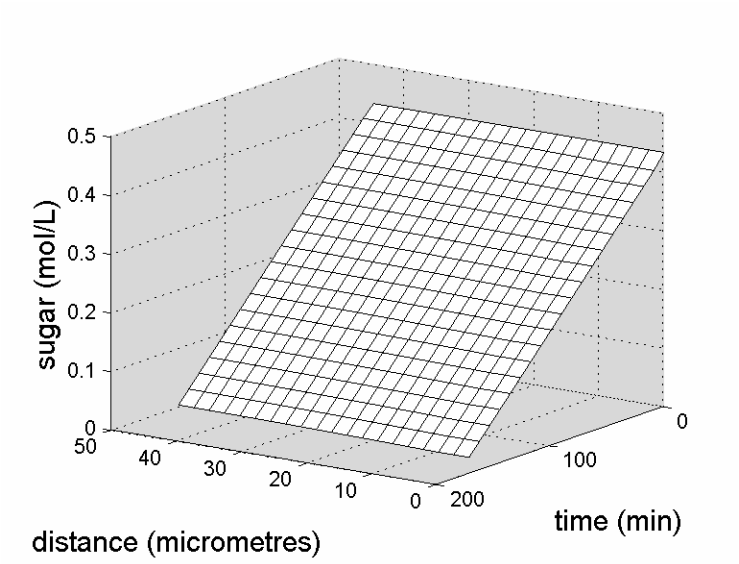
Simulation results showed that at any given time, concentration was the same at all locations (Figure 8.4). Sugar was exhausted after 167 minutes, and ethanol reached 0.78 $mol.L^{-1}$ at this time.

To test the sensitivity of results to the size of the modelling domain (L), the simulation was run with L decreased by 50% or increased by 100%.

Increasing L by 100% slightly slowed sugar consumption rate, but plots again showed equal sugar concentration in all locations at a given time. Diffusion appeared to 'smooth out' the local deficit of sugar and local excess of ethanol.

Figure 8.5. Effect of halving (top) and doubling (bottom) the space domain size (L) on simulated sugar concentration around a fermenting yeast cell.

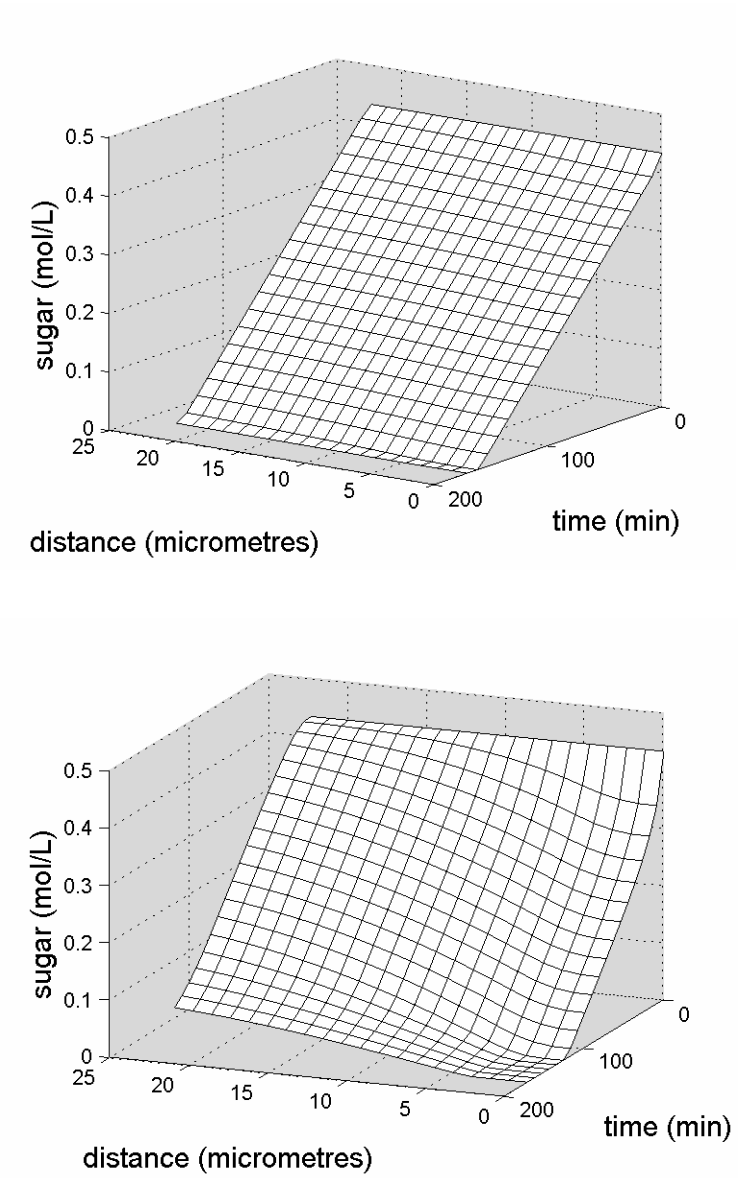




Top: modelling domain size (L) reduced by 50%, bottom: modelling domain increased by 100%.

There is considerable uncertainty in the estimated diffusion coefficients, and the Stokes-Einstein equation gives large errors in high-viscosity solutions (Cussler 1984). Sensitivity of simulation results to the value of diffusion coefficients was tested by running the simulation with coefficients artificially reduced a factor of 10 and 100 (Figure 8.6).

Figure 8.6. Effect of reducing diffusion coefficient by 10x (top) and 100x (bottom) on simulated sugar concentration around a fermenting yeast cell.



Slight curvature was introduced when D_g was decreased by a factor of 10, and curvature was pronounced with D_g reduced by 100 times.

Altering the sugar consumption rate (G_R), molar ethanol yield (Y_{eg}) and initial metabolite concentrations (g_i and s_i) between 80% and 120% of their original values (Table 8.1) altered the position of plots but not their shape.

8.3. Macrosystem model

A mathematical model is developed in this section to predict yeast metabolite dynamics during fermentation on the basis of experimental findings presented above and reports of sugar uptake kinetics in non-dough systems.

8.3.1 Model development

Sugar concentration in dough is the net result of (a) 'one-off' exogenous and endogenous sources, (b) dynamic sources comprising enzymatic hydrolysis reactions and (c) sugar consumption by yeast cells.

The only sugars consumed in significant quantities by yeast in fermenting dough are glucose, fructose, sucrose and maltose. Maltose was not consumed during 3-hour fermentations performed here (section 6.6), probably because glucose and fructose were not exhausted by the end of the fermentation.

This work has shown that the only hydrolysis reactions of practical importance were sucrose inversion and fructose liberation from unidentified oligosaccharides, and that these occurred only in yeasted doughs during mixing. Sucrose inversion was virtually complete by the end of mixing. Fructose liberation appeared to be minimal during fermentation, since ethanol production could be accounted for from sugars present by the end of mixing (section 6.8).

Sugars were not produced during fermentation, sucrose was hydrolysed during mixing and maltose was not consumed, so the model simply had to account for the uptake of glucose and fructose by yeast cells. Ethanol was also modelled because it is produced in large quantities in dough and may be injurious to yeast cells during the frozen dough process (section 1.9).

Describing ethanol flux is relatively straightforward, since no ethanol is supplied by ingredients and the only dynamic source is yeast metabolism. *Saccharomyces cerevisiae* will consume ethanol strictly aerobically (Walker 1998), but a mass balance between carbon dioxide and ethanol (section 6.9) suggested that reductive metabolism predominated in dough. Evaporation from the surface of dough would provide a dynamic ethanol sink, especially at a large surface area to volume ratio such as in small or flat doughs, but evaporation is neglected here.

Based on evidence in the literature and the results of experiments reported here, the following simplifying assumptions were made:

- Glucose and fructose are the only sugars consumed by yeast cells
- Sucrose is completely hydrolysed before fermentation begins
- Sugar uptake is the limiting step in sugar utilisation
- Cells do not accumulate sugars
- No dynamic sugar sources operate during fermentation
- Ethanol is not consumed by yeast cells or lost from the dough by evaporation
- Biomass is constant
- Concentrations are homogeneous

8.3.2 Mathematical treatment

Glucose and fructose uptake systems share much in common, and uptake rates of each are affected by the presence of the other (Orlowski and Barford 1987; D'Amore et al. 1989; Barford et al. 1992a). Several studies have observed that glucose is taken up in preference to fructose in liquid medium (Orlowski and Barford 1987; D'Amore et al. 1989; Barford et al. 1992a) and the same is seen in dough (Koch et al. 1954; Potus et al. 1994; Langemeier and Rogers 1995)). However few studies have examined in detail the interaction between glucose and fructose uptake in mixed media.

Barford et al. (1992a) discussed the interaction in terms of one or two active carrier types with differing specificity for either sugar. They simulated uptake by sugar-specific carriers with equation 8.27 and competitive or specific inhibition (Cornish-Bowden 1995) with equation 8.28. They also used equation 8.29, which is a special case of 8.28 in which $K_m = K_i$.

$$V = \frac{V_{max} X}{S_A + K_m} \qquad 8.27$$

$$V = \frac{V_{max} X}{S_A + K_m\left(1 + \frac{S_i}{K_i}\right)} = \frac{V_{max} X}{S_A + S_i (K_m / K_i) + K_m} \qquad 8.28$$

$$V = \frac{V_{max} X}{S_A + S_i + K_m} \qquad 8.29$$

V	rate of sugar uptake	[mmol.L^{-1}.h^{-1}]
V_{max}	maximum specific rate of sugar uptake	[mmol.(g biomass)$^{-1}$.h^{-1}]
X	biomass concentration	[g.L^{-1}]
S_A	concentration of substrate A	[mmol.L^{-1}]
S_i	concentration of inhibitor	[mmol.L^{-1}]
K_m	affinity constant	[mmol.L^{-1}]
K_i	competitive inhibition constant	[mmol.L^{-1}]

Barford et al. (1992a) incorporated uptake equations into an earlier model for aerobic growth (Barford and Hall 1981). They tested simulations with data from batch cultures growing on mixtures of glucose and fructose in aerobic conditions (Orlowski 1987). The most successful models for glucose uptake used combinations of 8.27 and 8.29, or 8.28 and 8.29. Fructose uptake was best simulated with 8.29 alone or in combination with 8.28.

Several combinations of the above models were used to simulate sugar uptake in doughs made with 2% added sucrose. Parameters were determined experimentally, taken from literature or adjusted for best fit within the range of values found by other workers.

Ethanol was predicted from the disappearance of glucose and fructose using molar yields (equation 8.30).

$$\frac{dE}{dt} = -Y_{eg}\frac{dG}{dt} - Y_{ef}\frac{dF}{dt} \qquad 8.30$$

E	ethanol concentration	[mol.L^{-1}]
G	glucose concentration	[mol.L^{-1}]
F	fructose concentration	[mol.L^{-1}]
Y_{eg}	molar yield of ethanol from glucose	-
Y_{ef}	molar yield of ethanol from fructose	-

8.3.3 Parameters

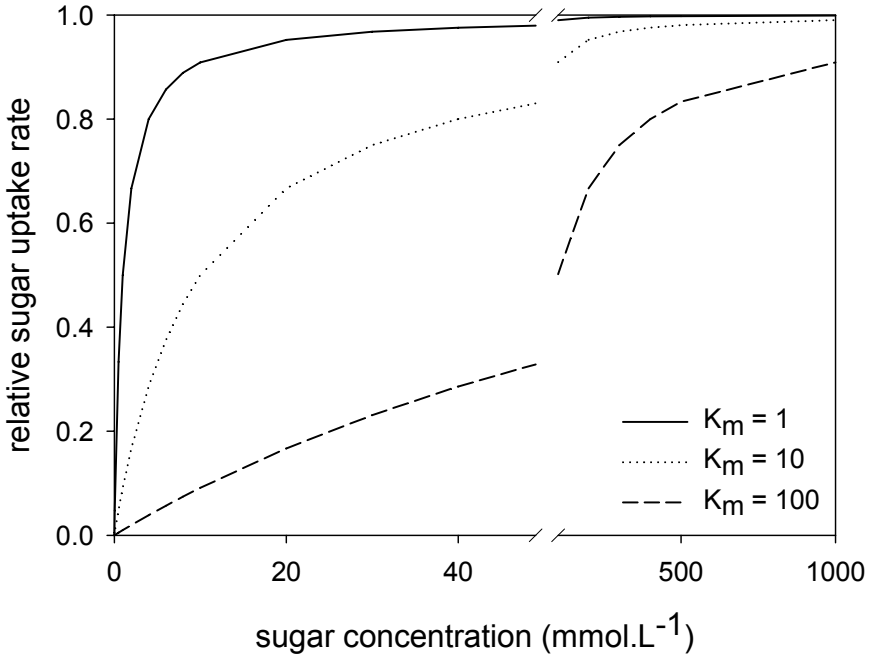
Uptake of sugars by yeast cells is facilitated by trans-membrane carrier systems, and for many sugars the dependence of uptake rate on sugar concentration follows hyperbolic kinetics (equation 8.27) over a wide range of sugar concentrations. However kinetic parameters for glucose and fructose uptake are quite different at low and high sugar concentration. At low concentration uptake is characterised by low K_m and is referred to as 'high-affinity' whereas at high concentration K_m is higher and 'low-affinity' uptake predominates. This may indicate multiple transport systems or interconversion between different forms of a single transport system (Serrano and De la Fuente 1974; Bisson and Fraenkel 1983; Lagunas 1993).

Sugar uptake can be monitored by measuring the accumulation of radioactivity in cultures exposed to radiolabeled sugars (Serrano and De la Fuente 1974; Bisson and Fraenkel 1983; D'Amore et al. 1989; Antuna and Martinez-Anaya 1993). The rate of uptake is measured at a range of sugar concentrations, and kinetic parameters determined by regression analysis.

Kinetic parameters are also determined in mathematical modelling studies. These may involve predictive models incorporating mechanistic equations, or descriptive models which fit or interpolate observations using empirical or semi-empirical equations (Roels

and Kossen 1978). Sugar uptake is highly sensitive to the value of the affinity constant (Figure 8.7).

Figure 8.7. Impact of the affinity constant, K_m on sugar uptake rate.



Relative rate is shown as a proportion of maximal rate.

Published values of K_m vary over four orders of magnitude for glucose (Table 8.2) and three orders of magnitude for fructose (Table 8.3), which emphasises their sensitivity to experimental conditions and calculation methods (Cornish-Bowden 1995).

Table 8.2. Published values of the affinity constant for glucose uptake.

Km (mmol.L^{-1})	*comment*	*source*
3	glucose concentration 2.5-20 mmol.L^{-1}	Serrano and De la Fuente (1974)
100	glucose concentration > 20 mmol.L^{-1}	
1.5 ± 0.25	glucose concentration < 10 mmol.L^{-1}	Bisson and Fraenkel (1983)
20 ± 8	glucose concentration > 10 mmol.L^{-1}	
1.6	glucose concentration 0.2-2.0 mmol.L^{-1}	D'Amore et al. (1989)
5.1	glucose concentration 2.5-100 mmol.L^{-1}	Antuna and Martinez-Anaya (1993)
0.5	predictive model	Barford et al. (1992a)
0.28	predictive model	Barford and Hall (1981)
0.53-0.61	predictive model	Peringer et al. (1974)
2.78	predictive model	Lafforgue-Delorme et al. (1994)
16.7	descriptive model	Lee et al. (1995)
0.56-2.76	descriptive model	Sonnleitner and Kapelli (1986)
2.76	descriptive model	Jarzebski et al. (1989)

Table 8.3. Published values of the affinity constant for fructose uptake.

K_m ($mmol.L^{-1}$)	*comment*	*source*
10	fructose concentration 2.5-20mmol.L^{-1}	Serrano and De la Fuente (1974)
100	fructose concentration > 20 mmol.L^{-1}	
6 ± 2	fructose concentration <10 mmol.L^{-1}	Bisson and Fraenkel (1983)
40 ± 15	fructose concentration >10 mmol.L^{-1}	
20	fructose concentration 0.2-2.0 mmol.L^{-1}	D'Amore et al. (1989)
6.5	fructose concentration 2.5-100 mmol.L^{-1}	Antuna and Martinez-Anaya (1993)
1	predictive model	Barford et al. (1992a)
53.8	predictive model	Smogrovicova et al. (2001)

Work by Serrano and De la Fuente (1974) and Bisson and Fraenkel (1983) examined the uptake of glucose and fructose alone over a wide range of concentrations, and with both sugars two regions of linearity were apparent in their results. High-affinity uptake (low K_m) occurred at sugar concentrations less than 10-20 mmol.L^{-1} and affinity constants were one or two orders of magnitude greater at higher sugar concentration. Other studies have not distinguished different kinetic regimes, and most mathematical models incorporate a single affinity constant. This is justified when the sugar concentration range of interest is considerably above the high-affinity region.

The region of primary interest here is 1-15 mmol.(100g dough)$^{-1}$. Aqueous phase water content was estimated at 24.0 ml.(100g dough)$^{-1}$ (section 4), therefore sugar concentration falls in the range 38.9-584 mmol.L^{-1} and low-affinity kinetics are appropriate.

Biomass concentration (X) in dough made with 2% compressed yeast was calculated from yeast dry matter (32.7% – Appendix A2) and solvent water in dough.

$$X = \frac{2\ g\ yeast}{100\ g\ dough} \cdot \frac{0.327\ g\ dry\ matter}{1\ g\ yeast} \cdot \frac{100\ g\ dough}{0.0257\ L\ water} \quad (8.3)_1$$

$$= 25.4\ g.L^{-1} \quad (8.3)_2$$

Maximal specific uptake rates of glucose and fructose ($V_{max\ G}$ and $V_{max\ F}$) were calculated from the rate at which they declined in doughs made with 2% of either sugar (section 6.7).

Initial glucose, fructose and ethanol levels (mmol.L^{-1}) were calculated from experimental gravimetric concentrations (mmol.(100g dough)$^{-1}$) measured immediately after mixing. Experimental yields of ethanol from glucose and fructose were 1.82 and 1.87 respectively (section 6.7). Other kinetic parameters were taken from Barford et al. (1992a).

Macrosystem model parameters are summarised in Table 8.4.

Table 8.4. Experimentally-determined parameters in the macro-scale model

symbol	*description*	*unit*	*value*
G_i	initial glucose concentration	$mol.L^{-1}$	0.250
F_i	initial fructose concentration	$mol.L^{-1}$	0.389
E_i	initial ethanol concentration	$mol.L^{-1}$	0.0559
X	biomass concentration	$g.L^{-1}$	25.4
Y_{eg}	molar yield of ethanol from glucose	-	1.82
Y_{ef}	molar yield of ethanol from fructose	-	1.87
$V_{max\ G}$	maximal glucose uptake velocity	$mol.(g\ biomass)^{-1}.h^{-1}$	0.00653
$V_{max\ F}$	maximal fructose uptake velocity	$mol.(g\ biomass)^{-1}.h^{-1}$	0.00626

8.3.4 Numerical Solution

A system of differential equations for sugar uptake and ethanol production (equations 8.33 to 8.36) was solved numerically using a program written in Matlab version 6.5 release 13 (The Mathworks Inc.). The program used a Matlab library algorithm (ODE45) based on an explicit Runge-Kutta numerical method. Program code is shown in Appendix A7.

$$\frac{dG}{dt} = -V_G \qquad 8.33$$

$$\frac{dF}{dt} = -V_F \qquad 8.34$$

$$\frac{dE}{dt} = -Y_{eg}\frac{dG}{dt} - Y_{ef}\frac{dF}{dt} \qquad 8.35$$

$$G, F, E, t > 0 \qquad 8.36$$

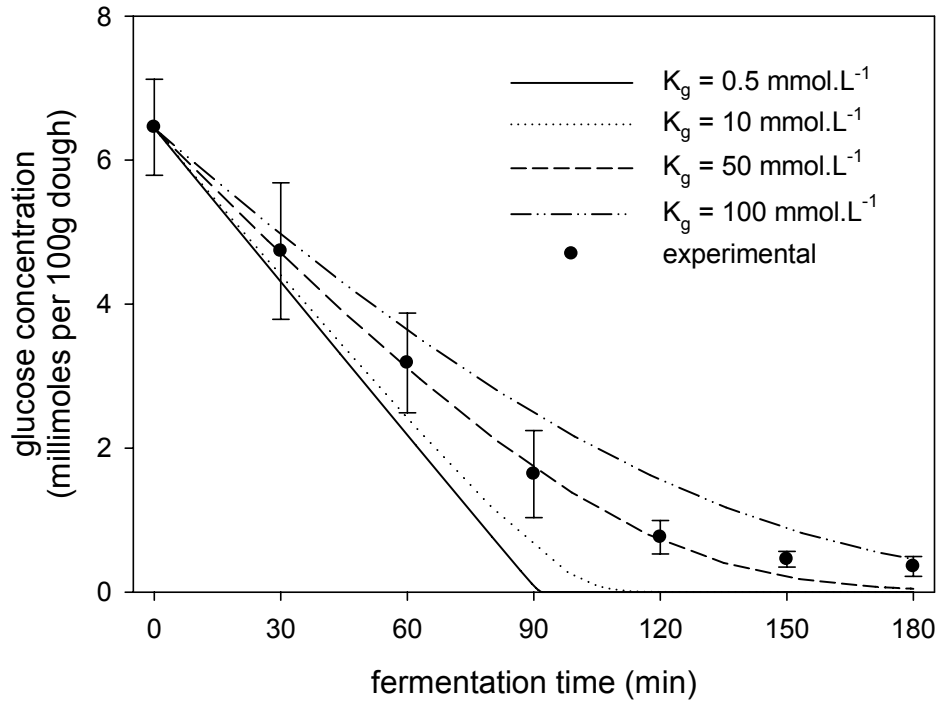
Various combinations of sugar uptake equations (8.27 to 8.29) and kinetic parameters were tested for accuracy of fit to experimental data.

8.3.5 Simulation Results

The simulation was required to predict glucose, fructose and ethanol in fermenting doughs made with 2% added sucrose. Simulation results were converted back from $mol.L^{-1}$ to $mmol.(100g\ dough)^{-1}$ for ease of comparison with experimental results.

Equation 8.27 was quite successful in predicting glucose uptake for the first 120 minutes of fermentation. It gave predictions slightly too low at 150 and 180 minutes (Figure 8.8). Best predictions were with affinity constant (K_g) at 50 $mmol.L^{-1}$, which is two orders of magnitude higher than the value used by Barford et al. (1992a) but within the range reported by other authors (Table 8.2).

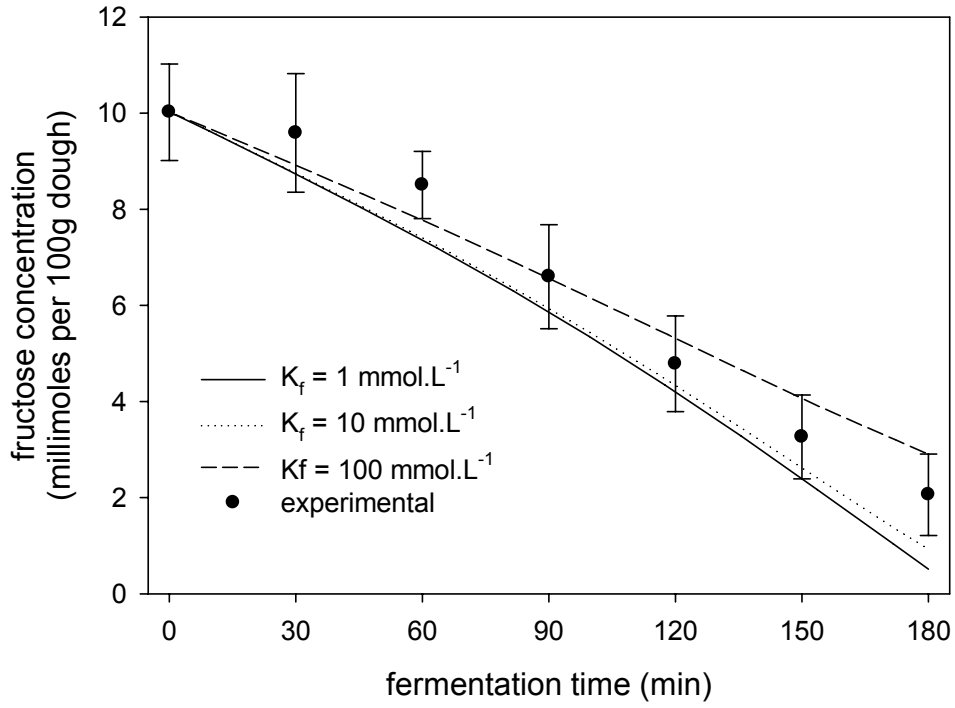
Figure 8.8. Glucose concentration in fermenting dough, measured or simulated with equation 8.27.



Doughs were made with 2% added sucrose and fermented at 30˚C. Glucose uptake was simulated with equation 8.27 using experimentally-determined initial concentration and maximal uptake rate, and a range of affinity constants (see legend). Vertical bars are standard deviations.

Baking strains of *Saccharomyces cerevisiae* take up glucose in preference to fructose (Koch et al. 1954; Potus et al. 1994). This rules out equation 8.27 for describing fructose uptake because the equation does not take glucose concentration into account. Equation 8.29 was trialled with K_f varying over three orders of magnitude (Figure 8.9).

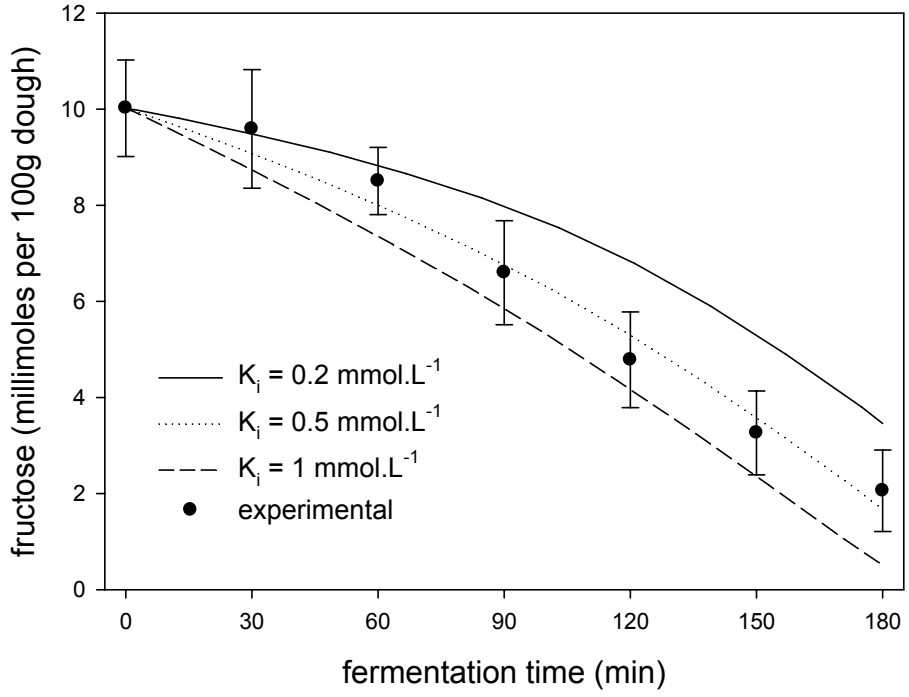
Figure 8.9. Fructose concentration in fermenting dough, measured or simulated with equation 8.29.



Doughs were made with 2% added sucrose and fermented at 30˚C. Fructose uptake was simulated with equation 8.29 using experimentally-determined initial concentration and maximal uptake rate, and a range of affinity constants (see legend). Vertical bars are standard deviations.

Equation 8.29 was unable to account for the delay in fructose consumption over the first hour of fermentation and predictions were generally poor. Equation 8.28 was trialled next, initially with K_m and K_i both set at 1 mmol.L^{-1} as reported by Barford et al. (1992a), then with other values of K_i (Figure 8.10). The data were simulated well when K_i was decreased to 0.5 mmol.L^{-1}

Figure 8.10. Fructose concentration in fermenting dough, measured or simulated with equation 8.28.



Doughs were made with 2% added sucrose and fermented at 30˚C. Fructose uptake was simulated with equation 8.28 using experimentally-determined initial concentration and maximal uptake rate, affinity constant K_m = 1 mmol.L^{-1} and a range of inhibition constants (K_i – see legend). and a range of affinity constants (see legend). Vertical bars are standard deviations.

In summary, the equations selected are:

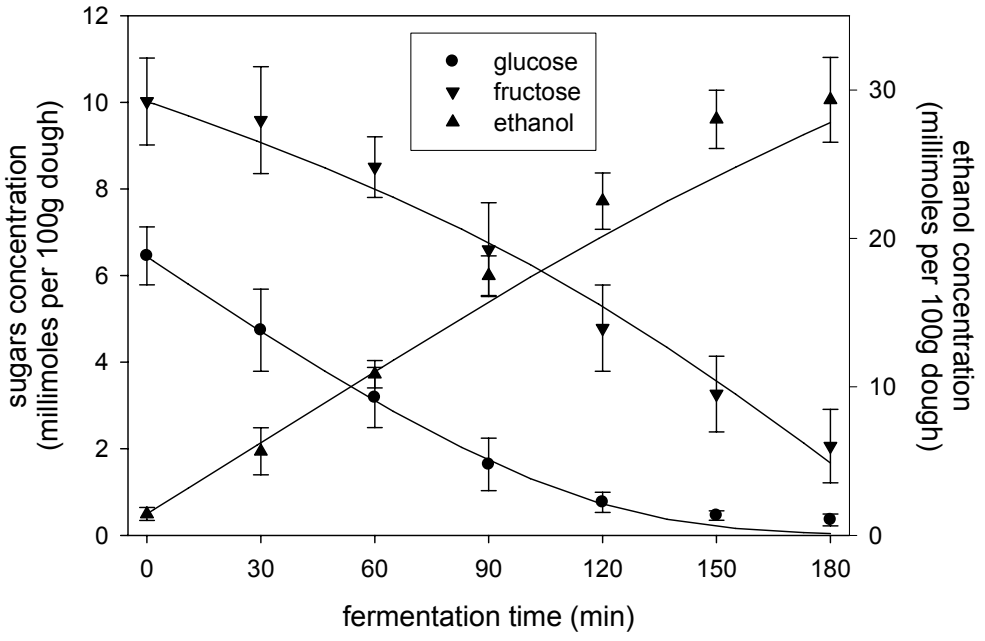
$$\frac{dG}{dt} = -\frac{V_{max\,G}\ X}{G + K_g} \qquad 8.37$$

$$\frac{dF}{dt} = \frac{V_{max\,F}\,X}{F + K_f\left(1 + \frac{G}{K_i}\right)} \qquad 8.38$$

$$\frac{dE}{dt} = -Y_{eg}\,\frac{dG}{dt} - Y_{ef}\,\frac{dF}{dt} \qquad 8.39$$

The macrosystem model predicted ethanol well for the first hour, but predictions were slightly too low at 90-150 minutes (Figure 8.11).

Figure 8.11. Simulated (lines) and experimental (points) glucose, fructose and ethanol concentrations in fermenting dough.



Doughs were made with 2% added sucrose and fermented at 30˚C. Sugars and ethanol were simulated with equations 8.37 to 8.39. Vertical bars are standard deviations.

8.4. Discussion

Although results showed that local gradients of yeast metabolites are unlikely, the dough may not be homogeneous on a larger scale. For example, any temperature gradients within the dough would theoretically affect the rate of sugar depletion and ethanol accumulation through their effect on yeast activity and the diffusion coefficient (equation 8.4).

The macrosystem model was very successful in fitting glucose concentration using a simple Michaelis-Menten kinetic equation. Although some workers have found that fructose inhibits glucose uptake (D'Amore et al. 1989), that appears not to be the case here, since no fructose term was necessary in the glucose equation.

An equation describing glucose as a competitive inhibitor of fructose uptake predicted fructose concentration in dough quite well. However it was unable to completely simulate the delay in fructose uptake in the first hour of fermentation while glucose was plentiful. The acceleration in fructose consumption in the second hour was consequently underestimated, and ethanol predictions were too low during this period.

It was not necessary to incorporate any initial delay in glucose consumption, confirming indications from section 6.9 that yeast cells had reached maximal activity after mixing and dividing. The assumption of constant biomass over the three hours did not appear to introduce substantial error into the simulation.

Lee et al. (1995) modelled the effect of ethanol on sugar uptake using an inhibition coefficient of the form:

$$\alpha = 1 - \frac{E}{E_{max}} \qquad 8.40$$

α	ethanol inhibition coefficient	-
E	ethanol concentration	[mol.L^{-1}]
E_{max}	maximum ethanol concentration at which sugar uptake occurs	[mol.L^{-1}]

This equation was trialled in the macrosystem model but did not improve the fit to experimental data.

8.5. Summary and conclusions

The microsystem model represents an idealised version of a single yeast cell consuming sugars and excreting ethanol during dough fermentation. It was designed to compare the timescale over which yeast metabolites are consumed or produced with the timescale over which they diffuse towards or away from the cell. Few of the parameters needed for the microsystem model were directly available in the baking literature, but it was possible to derive them from other systems. The macrosystem model was adapted from a kinetic model of aerobic batch fermentations.

Simulations showed no sugar or ethanol gradients developing around the cell because diffusion was faster than sugar consumption or ethanol production. The situation was unchanged when simulation parameters were altered within reasonable ranges either side of their estimated values. These results suggested that ethanol diffuses into the bulk aqueous phase as quickly as it is excreted and sugar uptake in dough is not diffusion-limited. The sugar and ethanol concentrations averaged over the whole dough are therefore likely to be representative of the concentrations at the surface of yeast cells.

The macrosystem model was very successful in predicting the kinetics of sugar consumption and ethanol accumulation in dough. Glucose strongly inhibited fructose uptake and fermentation was not inhibited by ethanol.

9. OVERALL SUMMARY AND CONCLUSIONS

The shelf life of frozen dough products is limited by a decline in gassing power, leading to unacceptably long proofing times. Industry experience has shown that best results are obtained by limiting the extent of pre-freezing fermentation, but the relationship between pre-fermentation time and shelf life is not linear. Several workers have reported that a pre-fermentation of 30-60 minutes is only moderately detrimental to post-thaw gassing power, and more prolonged fermentation causes a more rapid decrease in gassing power (Kline and Sugihara 1968; Meric et al. 1995; Nemeth et al. 1996; Almeida and Pais 1996; Kenny et al. 2001). A similar phenomenon was seen in this laboratory when dough was pre-fermented in bulk at room temperature (Shannon 2003).

It has been proposed that pre-fermentation renders yeast cells more vulnerable to freezing damage through depletion of cryo-protective solutes, most notably trehalose. A second hypothesis proposes that waste metabolites, especially volatiles like ethanol, accumulate during pre-fermentation and are then concentrated to injurious levels by freezing.

Baking research has historically been highly empirical, and although the optimal parameters for specific processes are well-researched there is still surprisingly little understanding of the biochemistry of dough fermentation. Few workers have measured ethanol in dough, in spite of its quantitative importance and putative role in freezing injury in frozen dough. Modern techniques for evaluating the metabolic state of yeast cells in dough (Autio and Mattila-Sandholm 1992) have not been exploited.

The objectives of this research were to:

- Define fluxes of the major yeast metabolites in dough during pre- and post-freezing fermentation.
- Mathematically model the uptake of substrate and production of ethanol by yeast in fermenting dough.
- Mathematically model the effect of yeast metabolism on the immediate environment of a yeast cell in fermenting dough.

The New Zealand compressed yeast used in this work, which is the only locally-manufactured product available, is considered by the manufacturer to be suitable for frozen dough applications. Preliminary work in this laboratory (Shannon 2003)

established that in the frozen dough system used here, gassing power over the first hour after thawing was quite sensitive to pre-fermentation time, e.g. doughs pre-fermented 90 minutes at 20˚C lost 50% of initial gassing power after storage at -20˚C for 48 hours.

The main yeast substrates in dough are glucose, fructose, sucrose and maltose. Yeast enzymes hydrolyse sucrose to glucose and fructose, both of which inhibit the uptake of maltose. Research with *Saccharomyces cerevisiae* in controlled batch fermentations and brewing worts has shown that glucose is consumed in preference to fructose, but maximal uptake rates of either sugar alone are similar, as are the yields of ethanol. Confirmatory experiments on doughs made with 2% glucose, fructose or sucrose showed no differences in gassing power or ethanol production. Yields of ethanol from glucose and fructose were 1.82 and 1.87 respectively.

Proofing power is inhibited by high levels of sugar or salt, and yeast activity is quite sensitive to the amount of water in the formulation. All doughs in this work were made with 33% water and 1% salt. In doughs made with added glucose, gassing power was optimal with 1-2% of this sugar.

Ethanol builds up to relatively high levels in fermenting dough, and the impact of exogenous ethanol on fermentation was measured. Doughs with 5 or 10 $ml.kg^{-1}$ added 95% ethanol produced gas rapidly at first, but maximal gassing rates were lower than doughs without ethanol. Ethanol production and sugar consumption were inhibited by 20 $ml.kg^{-1}$ added ethanol, but were equivalent with 0, 5 or 10 $ml.kg^{-1}$. Replication was insufficient to establish statistical rigour, as these experiments were of a confirmatory nature.

A rapid method for extracting yeast metabolites from dough was developed. The method involved freezing doughs in liquid nitrogen, grinding to a powder, homogenising the powder in water and filtering the homogenate. Extracts were analysed with enzymatic assays using a robotic transfer analyser. Recovery of metabolites from spiked doughs was good: ethanol, 99.7%; glucose, 92.4%; fructose, 100.5%; sucrose 106.6%; maltose 71.0%.

It was found that sugar concentrations in extracts fluctuated during storage at room temperature. The length of the delay between extraction and assay affected results, especially in fermented doughs. Extract stability was improved by heating at 85˚C for 1h, but extracts for ethanol assay were not heated to avoid evaporative losses. Sugar recoveries remained high in heated extracts: glucose, 94.1%; fructose 112.7%;

sucrose, 96.7%; maltose 92.2%. Fructose recovery above 100% was attributed to higher endogenous fructose in the spiked dough than in the control. Some doughs were re-extracted with heating and assayed again.

The method reported here for extracting sugars and ethanol is a significant advance over methods in the literature. It uses off-the-shelf equipment and reagents and is simpler and quicker than other methods. The extraction process is less prone to chemically modify dough components because dough structure is disrupted by physical means, i.e. freezing in liquid nitrogen and grinding to a powder, rather than prolonged extraction at elevated temperature in nonaqueous solvents.

The extraction method developed here will remove intact yeast cells, but a heat treatment is necessary to eliminate soluble enzymes. The heat treatment applied here, 85˚C for 1 hour, achieved this purpose but was probably longer and hotter than is strictly necessary.

The main sources of sugar in dough are endogenous flour sugars, refined sugars in the formulation and sugars liberated by enzymatic reactions such as starch hydrolysis. Flours were extracted and analysed using the same procedure as for doughs. Flour contained 0.23 mmol.100g^{-1} glucose, 0.17 mmol.100g^{-1} fructose, 2.74 mmol.100g^{-1} sucrose and 3.63 mmol.100g^{-1} maltose. These levels are quite similar to literature values.

In unyeasted doughs sucrose was constant throughout mixing and 3 hours of fermentation, but in yeasted doughs it fell 88% during mixing and declined to zero with subsequent fermentation. The drop in sucrose coincided with an equimolar rise in glucose, and fructose also rose sharply. The rise in fructose was greater than the drop in sucrose: an excess of 3.36 mmol.(100g dough)$^{-1}$ pointed to other sources of fructose such as fructan hydrolysis by yeast enzymes. Maltose increased slowly in both yeasted and unyeasted doughs, and was not consumed by yeast while glucose, fructose or sucrose were present.

This work has shown that in a simple dough system containing glucose, fructose or sucrose at levels typical of industrial formulations, maltose is not consumed and does not accumulate in substantial amounts over 3 hours at 30˚C; it can be largely ignored.

Mass balances between sugars (glucose, fructose and sucrose) and ethanol in fermenting dough were quite accurate in heated extracts. Carbon dioxide and ethanol were produced in equimolar quantities, indicating that yeast metabolised reductively.

At the time of writing, quantitative comparisons between carbon dioxide and ethanol in dough had not been reported in the literature. No experimental investigations of the oxidative status of yeast metabolism in fermenting dough had been published. The evidence reported here suggests that oxidative metabolism is quantitatively unimportant, which means that carbon dioxide production, as measured in a Risograph, is a good measure of ethanol production. Ethanol levels can be accurately estimated from simple manometric measurements, obviating the necessity to routinely extract and quantify ethanol in future research.

Sugars, ethanol and gassing power were measured in doughs pre-fermented 0, 30 or 90 minutes, frozen and stored at -18˚C for 48 h then thawed at 0˚C and fermented at 30˚C. Gassing power was diminished by pre-fermentation.

In doughs frozen, stored and thawed, mass balances were sometimes different from those in unfrozen dough. During post-thaw fermentation more carbon dioxide than ethanol was produced by unfermented and 30 minute pre-fermented doughs. This was attributed to dissolved CO_2 evaporating from doughs as they warmed from 0˚C to 30˚C, and evaporation was not related to yeast activity. The 90 minute pre-fermented doughs did not show this effect for unknown reasons.

Accumulation of ethanol corresponded with consumption of sugars in unfermented and 30 minute pre-fermented doughs, but exceeded sugar consumption in 90 minute pre-fermented doughs. This was probably an artifact of extraction without heating.

A mathematical model of a yeast cell metabolising in dough aqueous phase was constructed to compare the rates of sugar and ethanol diffusion with their consumption and production (respectively). In the model, diffusion occurred quickly enough that sugar taken up at the surface of the yeast cell was soon replaced by sugar diffusing in from surrounding aqueous phase. Ethanol diffused away from the cell quickly enough to prevent a buildup around the cell.

The simulatiuon results were very insensitive to changes in parameters, with no qualitative change when diffusion coefficients were varied over several orders of magnitude. The significance of this finding is that the average composition of the dough can be considered a reliable indication of the local conditions experienced by a yeast cell. Painstaking experimental measurements that would be required to measure metabolite concentrations at the surface of yeast cells in dough are completely unnecessary.

Yeast metabolites in a fermenting 2% sucrose dough were successfully predicted with a mathematical model based on enzyme kinetic equations. Glucose uptake followed simple Michaelis-Menten kinetics and fructose uptake was competitively inhibited by glucose. Experimental work showed that exogenous ethanol inhibited fermentation at high concentrations, but good predictions were obtained even when ethanol inhibition was not accounted for in the model. It was assumed that cell division during 3 hours of fermentation was negligible, and this did not seem to affect the accuracy of predictions.

This work has shown that that mathematical modeling methods derived in other microbial fermentations can be successfully applied to fermenting dough. It confirmed that *Saccharomyces cerevisiae* behaves the same in dough as in liquid broth with respect to preference between glucose and fructose. Further to this, modeling quantitatively explored the interaction between glucose and fructose uptake. It showed that glucose uptake behaved as if glucose-specific carriers were present and fructose uptake was inhibited by glucose in a 'competitive' way, i.e. the affinity was decreased (K_f increased) but the maximum specific fructose uptake velocity ($V_{max\ F}$) was unaffected.

The macro model in its entirety allows other researchers to accurately predict the amount of glucose, fructose and ethanol present in dough after a specified period of fermentation. Experimental measurements can be reliably interpolated, and once the model parameters have been established for any given dough system, routine sugar analysis will not be necessary.

10. RECOMMENDATIONS FOR FUTURE WORK

There is considerable uncertainty regarding the number of yeast cells actively metabolising in dough, the extent of cell division in dough and the effect of freezing and frozen storage on the metabolic state of cells. Freezing and frozen storage may kill or sub-lethally injure cells, but the extent of cell death or injury cannot be judged from current evidence.

Conventional plate counts do not correspond with gassing power, since the ability to form colonies on a plate is not directly linked with the ability to metabolise in dough. An *in situ* staining technique (Autio and Mattila-Sandholm 1992) has reportedly produced good results with dough and could be used to investigate the changes in yeast numbers and metabolic state through the frozen dough process.

Mixing and pre-fermentation at reduced temperature improves the shelf life of frozen dough. This has been explained in qualitative terms as reduced yeast activity at lower temperature. Future work should investigate the impact of fermentation temperature on sugar consumption and ethanol production, and whether the improved frozen shelf life of dough mixed at low temperature is related to reduced accumulation of fermentation wastes.

Water mobility in dough is likely to be altered during fermentation due to structural changes in gluten and the effect of yeast cell metabolism on dissolved solutes. Further work could examine whether fermentation produces measurable changes in water activity or water molecule relaxation states in dough.

Water movement in frozen dough appears to play a role in the loss of gas retention, but little is known about how it affects yeast cells. Research in other frozen food systems has pointed to the impact of molecular mobility and glass transition properties on storage stability, and future frozen dough research should examine the impact of pre-fermentation on such properties in dough.

This work has identified that aqueous dough extracts require a stabilising treatment to curb fluctuations in sugar concentration. It is recommended that a thermal or chemical stabilisation step is used in aqueous dough extractions.

The yeast used in this work was a New Zealand compressed yeast, which had different frozen dough performance to strains typically used for industrial frozen dough manufacture. Gassing power was more sensitive to pre-fermentation than is usually

reported. Different results may be obtained with specialised frozen dough yeasts manufactured in Europe or the US.

The microsystem and macrosystem models were not mathematically linked, but in the real system the diffusion of yeast metabolites in the vicinity of a yeast cell (microsystem) would influence concentration in the bulk phase (macrosystem). A similar situation exists in fluidised bed biofilm reactors. In these reactors small solid spheres coated with a biofilm are packed into a vertical column and a liquid substrate solution is pumped upwards through the column. Substrate diffuses into the biofilm where it is converted to product by the cells in the film, and product diffuses out into the bulk liquid phase. Parshotam et al. (1991) developed a mathematical modelling technique for linking diffusion and reaction kinetics in the biofilm with substrate and product concentrations in the bulk liquid phase. This could be used to link micro- and macro-system models of yeast cells in dough aqueous phase.

There is some evidence that yeast cells in different regions of a dough piece are affected differently by freezing (Le Bail et al. 1998; Havet et al. 2000). In the frozen dough process exterior regions are exposed to larger temperature gradients than inner regions, so they cool faster during freezing and warm faster during thawing. The impact of freezing rate on yeast cell viability is well-documented (Araki and Nei 1962; Mazur 1966; Mazur and Schmidt 1968; Mazur 1970).

A model for heat transfer in a dough piece during freezing and thawing could examine the occurrence of thermal gradients in dough. These might then be correlated with measures of yeast performance or molecular mobility at different locations within the dough and in doughs frozen under different regimes. Published data on the thermal properties of bakery products are readily available (Lind 1988; Lind 1991a; Lind 1991b; Kulp et al. 1995; Baik et al. 2001; Sablani et al. 2002).

LITERATURE CITED

Akdogan H, Ozilgen M. 1992. Kinetics of microbial growth gas production and dough volume increase during leavening. Enzyme and Microbial Technology 14(2):141-143.

Almeida MJ, Pais C. 1996. Leavening ability and freeze tolerance of yeasts isolated from traditional corn and rye bread doughs. Applied and Environmental Microbiology 62(12):4401-4404.

Amend T, Belitz HD. 1990. The formation of dough and gluten - a study by scanning electron microscopy. Zeitschrift fur Lebensmittel-Untersuchung und Forschung 190(5): 401-409.

American Association of Cereal Chemists. 1995. Approved methods of the American Association of Cereal Chemists. 9th ed. St. Paul, Minnesota: American Association of Cereal Chemists.

Antuna B, Martinez-Anaya MA. 1993. Sugar uptake and involved enzymatic activities by yeasts and lactic acid bacteria: their relationship with breadmaking quality. International Journal of Food Microbiology 18(3):191-200.

Arakawa T, Timasheff SN. 1982. Stabilization of protein structure by sugars. Biochemistry 21(25):6536-6544.

Araki T, Nei T. 1962. The mechanism of freezing in micro-organisms. I. Factors affecting the survival of yeast cells subjected to subzero temperatures. Low Temperature Science Series B(20):57-68.

Atkin L, Schultz AZ, Frey CN. 1945. Influence of dough constituents on fermentation. Cereal Chemistry 22:321-333.

Atkins PW, De Paula J. 2002. Physical chemistry. 7th ed. New York: W. H. Freeman.

Attfield PV, Kletsas S. 2000. Hyperosmotic stress response by strains of bakers' yeasts in high sugar concentration medium. Letters in Applied Microbiology 31(4):323-327.

Attfield PV, Raman A, Northcott CJ. 1992. Construction of Saccharomyces cerevisiae strains that accumulate relatively low concentrations of trehalose and their application in testing the contribution of the disaccharide to stress tolerance. FEMS Microbiology Letters 94(3):271-276.

Autio K, Laurikainen T. 1997. Relationship between flour/dough microstructure and dough handling and baking. Trends in Food Science and Technology 8:181-185.

Autio K, Mattila-Sandholm T. 1992. Detection of active yeast-cells (Saccharomyces-cerevisiae) in frozen dough sections. Applied and Environmental Microbiology 58(7):2153-2157.

Autio K, Sinda E. 1992. Frozen doughs: rheological changes and yeast viability. Cereal Chemistry 69(4):409-413.

Baguena R, Soriano MD, Martinez-Anaya MA, De Barber CB. 1991. Viability and performance of pure yeast strains in frozen wheat dough. Journal of Food Science 56(6):1690-1694, 1698.

Baier-Schenk A, Handschin S, Conde-Petit B. 2005. Ice in prefermented frozen bread dough - an investigation based on calorimetry and microscopy. Cereal Chemistry 82(3):251-255.

Baik OD, Marcotte M, Sablani SS, Castaigne F. 2001. Thermal and physical properties of bakery products. Critical Reviews in Food Science and Nutrition 41(5):321-352.

Barford JP. 1990. A general model for aerobic yeast growth: batch growth. Biotechnology and Bioengineering 35:907-920.

Barford JP, Hall RJ. 1981. A mathematical model for the aerobic growth of S. cerevisiae with a saturated respiratory capacity. Biotechnology and Bioengineering 23:1735-1762.

Barford JP, Phillips PJ, Orlowski JH. 1992a. A new model of uptake of multiple sugars by s. cerevisiae (part 1). Bioprocess Engineering 7(7):297-302.

Barford JP, Phillips PJ, Orlowski JH. 1992b. a new model of uptake of multiple sugars by S. cerevisiae (part 2). Bioprocess Engineering 7(7):303-307.

Batista AS, Miletti LC, Stambuk BU. 2004. Sucrose fermentation by Saccharomyces cerevisiae lacking hexose transport. Journal of Molecular Microbiology and Biotechnology 8(1):26-33.

Bekes F, Zawistowska U, Zillman RR, Bushuk W. 1986. Relationship between lipid content and compsition and loaf volume of twenty-six common spring wheats. Cereal Chemistry 63:327.

Bell BM, Daniels D.G.H., Fisher N. 1977. Physical aspects of the improvement of dough by fat. Food Chemistry 2(1):57-70.

Bender LD, Lamb J. 1977. The preservation of yeast viability in frozen dough. Journal of the Science of Food and Agriculture 28:952-953.

Beney L, Martinez de Maranon I, Marechal P-A, Gervais P. 2000. Influence of thermal and osmotic stresses on the viability of the yeast Saccharomyces cerevisiae. International Journal of Food Microbiology 55(1-3):275-279.

Benito B, Lagunas R. 1992. The low-affinity component of Saccharomyces-cerevisiae maltose transport is an artifact. Journal of Bacteriology 174(9):3065-3069.

Berglund PT, Shelton DR, Freeman TP. 1990. Comparison of two sample preparation procedures for low-temperature scanning electron microscopy of frozen bread dough. Cereal Chemistry 67(2):139-140.

Berglund PT, Shelton DR, Freeman TP. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. Cereal Chemistry 68(1):105-107.

Bergmeyer HU, Bergmeyer J, Grassl M, editors. 1984. Methods of enzymatic analysis. 3rd ed. Deerfield Beach, Florida: Verlag Chemie.

Berner JL, Gervais P. 1994. a new visualization chamber to study the transient volumetric response of yeast-cells submitted to osmotic shifts. Biotechnology and Bioengineering 43(2):165-170.

Berton B, Scher J, Villieras F, Hardy J. 2002. Measurement of hydration capacity of wheat flour: influence of composition and physical characteristics. Powder Technology 128(2-3):326-331.

Best D. 1995. Economic potential of frozen and refrigerated doughs and batters. In: Kulp K, Lorenz K, Brummer JM, editors. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists. p 1-18.

Bhattacharya M, Langstaff TM, Berzonsky WA. 2003. Effect of frozen storage and freeze-thaw cycles on the rheological and baking properties of frozen doughs. Food Research International 36:365-372.

Bisson L, Fraenkel DG. 1983. Involvement of kinases in glucose and fructose uptake

by Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America 80:1730-1734.

Blakebrough N, editor. 1967. Biochemical and biological engineering science. London: Academic Press.

Blanshard JMV, Lillford P, editors. 1993. The glassy state in foods. Loughborough, Leicestershire: Nottingham University Press.

Blish MF, Hughes RC. 1932. Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance. Cereal Chemistry 9:331-356.

Bloksma AH. 1971. Rheology and chemistry of dough. In: Pomeranz Y, Hlynka I, editors. Wheat chemistry and technology. 2nd ed. St. Paul, Minnesota: American Association of Cereal Chemists. p 523-584.

Bloksma AH. 1990a. Rheology of the breakmaking process. Cereal Foods World 35(2):237-244.

Bloksma AK. 1990b. Dough structure, dough rheology and baking quality. Cereal Foods World 35:237-244.

Blomberg A, Adler L. 1992. Physiology of osmotolerance in fungi. Advances in Microbial Physiology 33:145-212.

Blond G. 1985. Freezing in polymer-water systems and properties of water. In: Simatose D, Multon JL, editors. Properties of water in foods in relation to quality and stability. Proceedings of the NATO advanced research workshop on the influence of water on food quality and stability (ISOPOW 3). September 11-16, 1983, Beaune, France. Dordrecht: M. Nijhoff. p 531-54.

Blond G. 1988. Velocity of linear crystallization of ice in macromolecular systems. Cryobiology 25(1):61-66.

Bock MA. 2000. Minor constituents of cereals. In: Kulp K, Ponte JG, editors. Handbook of cereal science and technology. 2nd ed. New York: Marcel Dekker. p 479-504.

Bot A. 2003. Differential scanning calorimetric study on the effects of frozen storage on gluten and dough. Cereal Chemistry 80(4):366-370.

Bot A, De Bruijne DW. 2003. Osmotic properties of gluten. Cereal Chemistry 80(4):404-408.

Bruinsma BL, Giesenschlag J. 1984. Frozen dough performance. compressed yeast - instant dry yeast. Baker's Digest 58(6):6-7, 11.

Budiaman ER, Fennema OR. 1987a. Linear rate of water crystallization as influenced by temperature of hydrocolloid suspensions. Journal of Dairy Science 70(3):534-546.

Budiaman ER, Fennema OR. 1987b. Linear rate of water crystallization as influenced by viscosity of hydrocolloid suspensions. Journal of Dairy Science 70(3):547-554.

Bushuk W. 1966. Distribution of water in dough and bread. Baker's Digest 40(5):38-40.

Bushuk W. 1998. Interactions in wheat doughs. In: Hamer R, Hoseney RC, editors. Interactions: the keys to cereal quality. St. Paul, Minnesota: American Association of Cereal Chemists. p 1-16 .

Bushuk W, Mehrotra VK. 1977a. Studies of water binding by differential thermal analysis. I. dough studies using the boiling mode. Cereal Chemistry 54(2):311-320.

Bushuk W, Mehrotra VK. 1977b. Studies of water binding by differential thermal analysis. II. dough studies using the melting mode. Cereal Chemistry 54(2):320-325.

Bushuk W, Winkler CA. 1957. Sorption of water vapor on wheat flour, starch, and gluten. Cereal Chemistry 34(2):73-86.

Busturia A, Lagunas R. 1985. Identification of two forms of the maltose transport system in Saccharomyces-cerevisiae and their regulation by catabolite inactivation. Biochimica et Biophysica Acta 820(2):324-326.

Busturia A, Lagunas R. 1986. Catabolite inactivation of the glucose transport system in Saccharomyces-cerevisiae. Journal of General Microbiology 132(2):379-386.

Carpenter JF, Crowe JH. 1989. An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry 28(9):3916-3922.

Cartwright CP, Juroszek JR, Beavan MJ, Ruby FMS, Demorais SMF, Rose AH. 1986. Ethanol dissipates the proton-motive force across the plasma- membrane of Saccharomyces-cerevisiae. Journal of General Microbiology 132:369-377.

Casey GP, Foy JJ. 1995. Yeast performance in frozen doughs and strategies for

improvement. In: Kulp K, Lorenz K, Brummer JM, editors. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists. p 19-52.

Champion D, Le Meste M, Simatos D. 2000. Towards an improved understanding of glass transition and relaxations in foods: molecular mobility in the glass transition range. Trends in Food Science and Technology 11(2):41-55.

Chen SL, Chiger M. 1985. production of baker's yeast. In: Moo-Young M, editor-in-chief. Comprehensive biotechnology : the principles, applications, and regulations of biotechnology in industry, agriculture, and medicine. Volume 3. Oxford: Pergamon. p 430-461.

Cherian G, Chinachoti P. 1996. 2H and 17O Nuclear magnetic resonance study of water in gluten in the glassy and rubbery state. Cereal Chemistry 73(5):618-624.

Chiotellis E, Campbell GM. 2003a. Proving of bread dough I. Modelling the evolution of the bubble size distribution. Food and Bioproducts Processing 81(3):194-206.

Chiotellis E, Campbell GM. 2003b. Proving of bread dough II. Measurement of gas production and retention. Food and Bioproducts Processing 81(3):207-216.

Chung OK, Pomeranz Y, Finney KF. 1982. Relation of polar lipid content of mixing requirement and loaf volume potential of hard red winter wheat flour. Cereal Chemistry 59(1):14-20.

Chung OK, Pomeranz Y, Jacobs RM, Howard BG. 1980. Lipid extraction conditions to differentiate among hard red winter wheats that vary in breadmaking. Journal of Food Science 45(5):1168-1174.

Clegg JS, Seitz P, Seitz W, Hazlewood CF. 1982. Cellular response to extreme water loss: the water-replacement hypothesis. Cryobiology 19:306-316.

Collins B. 1985. Breadmaking processes. In: National Association of Master Bakers (Great Britain). the master bakers' book of breadmaking. 2nd ed. Ware, Hertfordshire, UK: National Association of Master Bakers. p 1-46.

Coons DM, Boulton RB, Bisson LF. 1995. Computer-assisted nonlinear-regression analysis of the multicomponent glucose-uptake kinetics of Saccharomyces-cerevisiae. Journal of Bacteriology 177(11):3251-3258.

Cooper EJ, Reed G. 1968. Yeast fermentation - effects of temperature, pH, ethanol, sugars, salt and osmotic pressure. Bakers' Digest 42(6):22-24, 26, 28-29, 63.

Cornish-Bowden A. 1995. Fundamentals of enzyme kinetics. London: Portland press.

Crowe JH, Carpenter JF, Crowe LM, Anchordoguy TA. 1991. Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology 27:219-231.

Crowe JH, Crowe LM. 1982. Induction of anhydrobiosis: membrane changes during drying. Cryobiology 19:317-328.

Crowe JH, Hoekstra FA, Crowe LM. 1992. Anhydrobiosis. Annual Review of Physiology 54:579-599.

Crowe LM, Reid DS, Crowe JH. 1996. Is trehalose special for preserving dry biomaterials? Biophysical Journal 71(4):2087-2093.

Crumplen RM, Slaughter JC, Stewart GG. 1996. Characteristics of maltose transporter activity in an ale and lager strain of the yeast Saccharomyces cerevisiae. Letters in Applied Microbiology 23(6):448-452.

Cussler EL. 1984. Diffusion, mass transfer in fluid systems. Cambridge: Cambridge University Press.

D'Amore T, Crumplen R, Stewart GG. 1991. The involvement of trehalose in yeast stress tolerance. Journal of Industrial Microbiology 7:191-196.

D'Amore T, Russell I, Stewart GG. 1989. Sugar utilization by yeast during fermentation. Journal of Industrial Microbiology 4(4):315-324.

D'Amore T, Stewart GG. 1987. Ethanol tolerance of yeast. Enzyme and Microbial Technology 9(6):322-330.

D'Appolonia BL, Gilles KA, Osman EM, Pomeranz Y. 1971. Carbohydrates. In: Pomeranz Y, Hlynka I, editors. Wheat chemistry and technology. 2nd ed. St. Paul, Minnesota: American Association of Cereal Chemists. p 301-92.

Daniels NWR. 1975. Some effects of water in wheat flour doughs. In: Duckworth RB, editor. Water relations of foods: proceedings of an international symposium. September 1974, Glasgow. London: Academic Press. p 573-86.

Davies RJ, Daniels NWR, Greenshields RN. 1969. An improved method of adjusting flour moisture in studies on lipid binding. Journal of Food Technology 4(2):117-123.

Davies RJ, Webb T. 1969. Calorimetric determination of freezable water in dough. Chemistry and Industry 1969(33):1138-1139.

De Graaf E, Madeka H, Cocero AM, Kokini J. 1993. Determination of the effect of moisture on glass gliadin transition using mechanical spectrometry and differential scanning calorimetry. Biotechnology Progress 9:210-213.

Dombek KM, Ingram LO. 1987. Ethanol-production during batch fermentation with Saccharomyces-cerevisiae - changes in glycolytic-enzymes and internal pH. Applied and Environmental Microbiology 53(6):1286-1291.

Dowd JE, Riggs DS. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. Journal of Biological Chemistry 240(2):863-869.

Dubois DK, Blockolsky D. 1986. Frozen bread dough. Effect of dough mixing and thawing methods. American Institute of Baking Technical Bulletin 8(6):1-7.

Dunas F. 1988. Screening for a yeast strain for frozen bread doughs. In: Asp N-G, editor. Cereal science and technology in Sweden. Lund, Sweden: University of Lund. p 130-136.

El-Hady EA, El-Samahy SK, Seibel W, Brümmer JM. 1996. changes in gas production and retention in non-prefermented frozen wheat doughs. Cereal Chemistry 73(4):472-477.

Eleutherio ECA, Araujo PS, Panek AD. 1993. Protective role of trehalose during heat stress in Saccharomyces cerevisiae. Cryobiology 30(6):591-596.

Eliasson AC, Larsson K. 1993. Cereals in breadmaking: a molecular colloidal approach. New York: Marcel Dekker.

Erickson MC, Hung Y-C. 1997. Quality in frozen food. New York: Chapman & Hall.

Ernandes JR, D'Amore T, Russell I, Stewart G. 1992. Regulation of glucose and maltose transport in strains of Saccharomyces. Journal of Industrial Microbiology 9(2):127-130.

Ernandes JR, Williams JW, Russell I, Stewart GG. 1993. Effect of yeast adaptation to maltose utilization on sugar uptake during the fermentation of brewers wort. Journal of the Institute of Brewing 99(1):67-71.

Esselink EFJ, Van Aalst H, Maliepaard M, Van Duynhoven JPM. 2003. Long-term storage effect in frozen dough by spectroscopy and microscopy. Cereal Chemistry 80(4):396-403.

Evans IH. 1990. Yeast strains for baking: recent developments. In: Spencer JFT, Spencer DM, editors. yeast technology. New York: Springer-Verlag. p 13-54.

Farrand EA. 1969. Starch damage and alpha-amylase as bases for mathematical models relating to flour water-absorption. Cereal Chemistry 46(2):103-116.

Fausett LV. 1999. Applied numerical analysis. East Rutherford, New Jersey: Prentice Hall.

Felske LV, Silva RF, inventors; Stauffer Chemical Company, assignee. 1988 May 10. Method for producing frozen yeast-leavened dough. United States Patent 4 743 452.

Fennema OR, Powrie WD. 1964. Fundamentals of low-temperature food preservation. Advances in Food Research 13:219.

Fennema OR. 1973. Nature of the freezing process. In: Fennema OR, Powrie WD, Marth EH, editors. Low-temperature preservation of foods and living matter. New York: Marcel Dekker. p 151-241.

Fennema OR. 1996. Water and ice. In: Fennema OR, editor. Food chemistry. 3rd ed. New York: Marcel Dekker. p 17-94.

Finney PL, Bains GS. 1999. Protein functionality differences in eastern us soft wheat cultivars and interrelation with end-use quality tests. Lebensmittel Wissenschaft und Technologie 32(7):406-415.

Franks F, editor. 1982. Water and aqueous solutions at subzero temperatures . In: Franks F, editor. Water, a comprehensive treatise. Volume 7. New York: Plenum Press

Franks F. 1985. Biophysics and biochemistry at low temperatures. Cambridge: Cambridge University Press.

Frasse P, Lambert S, Richard-Molard D, Chiron H. 1993. The influence of fermentation on volatile compounds in french bread dough. Lebensmittel Wissenschaft und Technologie 26:126-132.

Fuhrmann GF, Volker B. 1992. Regulation of glucose-transport in Saccharomyces-cerevisiae. Journal of Biotechnology 27(1):1-15.

Fuhrmann GF, Volker B. 1993. Misuse of graphical analysis in nonlinear sugar-transport kinetics by Eadie-Hofstee plots. Biochimica Et Biophysica Acta 1145(1):180-182.

Fujio Y, Lim J-K. 1989. Correlation between the glass-transition point and color change of heat-treated gluten. Cereal Chemistry 66(4):268-270.

Gadd GM, Chalmers K, Reed RH. 1987. The role of trehalose in dehydration resistance of Saccharomyces cerevisiae. FEMS Microbiology Letters 48(1/2):249-254.

Gamo FJ, Moreno E, Lagunas R. 1995. The low-affinity component of the glucose-transport system in Saccharomyces-cerevisiae is not due to passive diffusion. Yeast 11(14):1393-1398.

Gancedo C, Serrano R. 1989. Energy-yielding metabolism. In: Rose AH, Harrison JS, editors. the yeasts. 2nd ed. Volume 3. London: Academic Press. p 205-60.

Gelinas P, Deaudelin I, Grenier M. 1995. Frozen dough: effects of dough shape, water-content, and sheeting-molding conditions. Cereal Foods World 40(3):124-126.

Gelinas P, Fiset G, Le Duy A, Goulet J. 1989. Effect of growth conditions and trehalose content on cryotolerance of bakers' yeast in frozen doughs. Applied and Environmental Microbiology 55(10):2453-2459.

Gelinas P, Lagimoniere M, Rodrigue N. 1994. Performance of cream or compressed yeast in frozen and nonfrozen doughs. Cereal Chemistry 71(2):183-186.

George RM. 1997. Freezing systems. In: Erickson MC, Hung Y-C. Quality in frozen food. New York: Chapman & Hall. p 3-9.

Gervais P, Marechal P. 1994. Yeast resistance to high levels of osmotic pressure: influence of kinetics. Journal of Food Engineering 22(1-4):399-407.

Giannou V, Kessoglou V, Tzia C. 2003. Quality and safety characteristics of bread

made from frozen dough. Trends in Food Science and Technology 14(3):99-108.

Girhammar U, Asp N-G, Nair BM. 1988. Certain physical and physicochemical characteristics of cereal pentosans and their effect on dough and bread . In: Asp N-G, editor. Cereal science and technology in Sweden. Lund, Sweden: University of Lund. p 249-254.

Godkin W, Cathcart W. 1949. Fermentation activity and survival of yeast in frozen and unfermented doughs. Food Technology 3:139.

Goedeken DL, Lonergan DA, Sierzant RL, inventors; The Pillsbury Company, assignee. 2003 Dec 9. Pre-proofed freezer-to-oven dough compositions, and methods. United States Patent 6 660 311 .

Greer EN, Stewart BA. 1959. The water absorption of wheat flour: relative effects of protein and starch. Journal of the Science of Food and Agriculture 10:248.

Gutierrez C, Abee T, Booth IR. 1995. Physiology of the osmotic stress response in microorganisms. International Journal of Food Microbiology 28:233-244.

Harrison JS. 1970. Yeast production. Progress in Industrial Microbiology 10:129-177.

Hatley RHM, Van den Berg C, Franks F. 1991. The unfrozen water content of maximally freeze concentrated carbohydrate solutions: validity of the methods used for its determination. Cryo Letters 12(2):113-124.

Hautera P, Loevgren T. 1975. Alpha-glucosidase, alpha-glucoside permease, maltose fermentation and leavening ability of baker's yeast. Journal of the Institute of Brewing 81(4):309-313.

Havet M, Mankai M, Le Bail A. 2000. Influence of the freezing condition on the baking performances of french frozen dough. Journal of Food Engineering 45(3):139-145.

Heckelmann U. 1993. Gaerzeitsteuerung. Verfahrenstechnik von heute und morgen. Brot und Backwaren 41(12):34-36.

Hibberd GE, Parker NS. 1976. Gas pressure-volume-time relationships in fermenting doughs. I. Rate of production and solubility of carbon dioxide in dough. Cereal Chemistry 53(3):338-346.

Hills BP, Cano C, Belton PS. 1991. Proton NMR relaxation studies of aqueous

polysaccharide systems. Macromolecules 24(10):2944-2950.

Hills BP, Takacs SF, Belton PS. 1990. A new interpretation of proton NMR relaxation time measurements of water in food. Food Chemistry 37(2):95-112.

Hino A, Mihara K, Nakashima K, Takano H. 1990. Trehalose levels and survival ratio of freeze-tolerant versus freeze-sensitive yeasts. Applied and Environmental Microbiology 56(5):1386-1391.

Hino A, Takano H, Tanaka Y. 1987. New freeze-tolerant yeast for frozen dough preparations. Cereal Chemistry 64(4):269-275.

Hoffman C, Schweitzer TR, Dalby G. 1941. Factors affecting the growth of yeast in fermenting doughs. Cereal Chemistry 18:342-349.

Hoseney RC, Finney KF, Pomeranz Y, Shogren MD. 1971. Functional (breadmaking) and biochemical properties of wheat flour components. VIII. Starch. Cereal Chemistry 48(2):191-201.

Hoseney RC, Zeleznak K, Lai CS. 1986. Wheat gluten: a glassy polymer. Cereal Chemistry 62(3):285-286.

Hsu KH, Hoseney RC, Seib PA. 1979a. Frozen dough. I. Factors affecting stability of yeasted doughs. Cereal Chemistry 56(5):419-424.

Hsu KH, Hoseney RC, Seib P.A. 1979b. Frozen dough. II. Effects of freezing and storing conditions on the stability of yeasted doughs. Cereal Chemistry 56(5):424-426.

Ingram LO, Buttke TM. 1984. Effects of alcohols on micro-organisms. Advances in Microbial Physiology 22:253-300.

Inoue Y, Bushuk W. 1991. Studies on frozen doughs. I. Effects of frozen storage and freeze-thaw cycles on baking and rheological properties. Cereal Chemistry 68(6):627-631.

Inoue Y, Bushuk W. 1992. Studies on frozen doughs. II. Flour quality requirements for bread production from frozen dough. Cereal Chemistry 69(4):423-428.

Inoue Y, Bushuk W. 1996. Effects of freezing, frozen storage and thawing on dough and baked goods. In: Jeremiah LE, editor. Freezing effects on food quality. New York: Marcel Dekker. p 367-400.

Inoue Y, Sapirstein HD, Takayanagi S, Bushuk W. 1994. Studies on frozen doughs. III. Some factors involved in dough weakening during frozen storage and thaw-freeze cycles. Cereal Chemistry 71(2):118-121.

Izidorczyk M, Biliaderis CG, Bushuk W. 1991. Physical properties of water-soluble pensotsans from different wheat varieteis. Cereal Chemistry 68:145-50.

James AM, Lord MP. 1992. Macmillan's chemical and physical data. London: Macmillan.

Jarzebski AB, Malinowski JJ, Goma G. 1989. Modeling of ethanol fermentation at high yeast concentrations. Biotechnology and Bioengineering 34:1225-1230.

Jenkins SM. 1975. Bakery technology. Toronto: Lester and Orpen.

Jeremiah LE, editor. 1996. Freezing effects on food quality. New York: Marcel Dekker.

Jones RP. 1987a. Measures of yeast death and deactivation and their meaning. I. Process Biochemistry 22(1):118-128.

Jones RP. 1987b. Use of iso-osmolality plots to characterize yeast fermentative performance. Journal of Applied Bacteriology 62(4):349-360.

Jones RP, Greenfield PF. 1986. Role of water activity in ethanol fermentations. Biotechnology and Bioengineering 28(1):29-40.

Jones RP, Greenfield PF. 1987. Specific and non-specific inhibitory effects of ethanol on yeast growth. Enzyme and Microbial Technology 9(6):334-338.

Kenny S, Grau H, Arendt EK. 2001. Use of response surface methodology to investigate the effects of processing conditions on frozen dough quality and stability. European Food Research and Technology 213(4-5):323-328.

Kline L, Sugihara TF. 1968. Factors affecting the stability of frozen bread doughs. I - Prepared by the straight dough method. Baker's Digest 42(5):44-46, 48-50.

Koch RB, Smith F, Geddes WF. 1954. The fate of sugars in bread doughs and synthetic nutrient solutions undergoing fermentation with baker's yeast. Cereal Chemistry 31(2):55-72.

Kokini JL, Cocero AM, Madeka H, De Graaf E. 1994. The development of state diagrams for cereal proteins. Trends in Food Science and Technology 5:281-288.

Kou Y, Ross EW, Taub IA. 2002. Microstructural domains in foods: effect of constituents on the dynamics of water in dough, as studied by magnetic resonance spectroscopy. In: Levine H, editor. Amorphous food and pharmaceutical systems. Cambridge: Royal Society of Chemistry. p 49-58.

Kulp K, Lorenz K, Brümmer JM, editors. 1995. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists.

Kulp K, Ponte JG. 2000. Breads and yeast-leavened bakery products. In: Kulp K, Ponte JG. Handbook of cereal science and technology. 2nd ed. New York: Marcel Dekker. p 539-74.

Kulp K. 1995. Biochemical and biophysical principles of freezing. In: Kulp K, Lorenz K, Brummer JM, editors. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists. p 63-89.

Kurz T, Mieleitner J, Becker T, Delgado A. 2002. A model based simulation of brewing yeast propagation. Journal of the Institute of Brewing 108:248-255.

Laaksonen T, Labuza T. 2001. Effects of moisture, sucrose, NaCl and arabinoxylan on relaxation in wheat dough as measured by DMTA. International Journal of Food Properties 4(2):311-325.

Laaksonen TJ, Roos YH. 2000. Thermal, dynamic-mechanical, and dielectric analysis of phase and state transitions of frozen wheat doughs. Journal of Cereal Science 32(3):281-292.

Laaksonen TJ, Roos YH. 2001a. Dielectric relaxations of frozen wheat doughs containing sucrose, NaCl, ascorbic acid and their mixtures. Journal of Cereal Science 33(3):331-340.

Laaksonen TJ, Roos YH. 2001b. Thermal and dynamic-mechanical properties of frozen wheat doughs with added sucrose, NaCl, ascorbic acid, and their mixtures. International Journal of Food Properties 4(2):201-213.

Laaksonen TJ, Roos YH. 2003. Water sorption and dielectric relaxations of wheat dough (containing sucrose, NaCl, and their mixtures). Journal of Cereal Science 37:319-326.

Lafforgue-Delorme C, Delorme P, Goma G. 1994. Continuous alcoholic fermentation

with Saccharomyces cerevisiae recycle by tangential filtration: key points for process modelling. Biotechnology Letters 16(7):741-746.

Lagunas R. 1993. Sugar transport in Saccharomyces-cerevisiae. Fems Microbiology Reviews 104(3-4):229-242.

Langemeier JM, Rogers D. 1995. Rapid method for sugar analysis of doughs and baked products. Cereal Chemistry 72(4):349-351.

Larsen NG, Humphrey-Taylor VJ, Baruch DW. 1989. Glycolipid content as a breadmaking quality determinant in flours from New Zealand wheat blends. Journal of Cereal Science 9(2):149-157.

Larsen RA. 1964. Hydration as a factor in bread flour quality. Cereal Chemistry 41:181.

Larsson H. 2002. Effect of pH and sodium chloride on wheat flour dough properties: ultracentrifugation and rheological measurements. Cereal Chemistry 79(4):544-545.

Larsson H, Eliasson AC. 1996a. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation.1. Influence of water content. Cereal Chemistry 73(1):18-24.

Larsson H, Eliasson AC. 1996b. Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. 2. Influence of mixing time, ascorbic acid, and lipids. Cereal Chemistry 73(1):25-31.

Lasztity R. 1996. Cereal proteins. 2nd ed. Boca Raton: CRC Press.

Le Bail A, Grinand C, Le Cleach S, Martinez S, Quilin E. 1999. Influence of storage conditions on frozen french bread dough. Journal of Food Engineering 39(3):289-291.

Le Bail A, Havet M, Pasco M. 1998. Influence of the freezing rate and of storage duration on the gassing power of frozen bread dough. In: Hygiene, quality and safety in the cold chain and air conditioning. 1998, Nantes, France. Paris: International Institute of Refrigeration. p 181-188

Le Bail A, Havet M, Pasco M, Chourot JM. 1997. Vietesse de congelation et qualite. application a la congelation des pates a pain crues. Revue Generale du Froid 978:20-24.

Le Meste M, Champion D, Roudaut G, Blond G, Simatos D. 2002. Glass transition and

food technology: a critical appraisal. Journal of Food Science 67(7):2444-2458.

Leao C, Van Uden N. 1982. Effects of ethanol and other alkanols on the glucose-transport system of Saccharomyces-cerevisiae. Biotechnology and Bioengineering 24(11):2601-2604.

Lee FA. 1970. The effects of bound and available water on enzymic processes in wheat flour doughs. Food Technology in Australia 22(9):516-20.

Lee JW, Cuendet LS, Geddes WF. 1959. The fate of various sugars in fermenting sponges and doughs. Cereal Chemistry 36:522-533.

Lee JW, Geddes WF. 1959. The role of amylase activity in the rapid increase of the maltose content of wheat flour doughs during mixing. Cereal Chemistry 36:554-558.

Lee S, Cornillon P, Kim YR. 2002. Spatial investigation of the non-frozen water distribution in frozen foods using NMR SPRITE. Journal of Food Science 67(6):2251-2255.

Lee SY, Pyrak-Nolte LJ, Campanella O. 2004. Determination of ultrasonic-based rheological properties of dough during fermentation. Journal of Texture Studies 35(1):33-51.

Lee Y-S, Lee WG, Chang YK, Chang HN. 1995. Modelling of ethanol production by Saccharomyces cerevisiae from a glucose and maltose mixture. Biotechnology Letters 17(8):791-796.

Lefebvre D, Gabriel V, Vayssier Y, Fontagne-Faucher C. 2002. Simultaneous HPLC determination of sugars, organic acids and ethanol in sourdough process. Lebensmittel Wissenschaft und Technologie 35(5):407-414.

Lehmann TA, Dreese P. 1981. Stability of frozen bread dough - effects of freezing temperatures. American Institute of Baking Technical Bulletin 3(7):1-5.

Leung HK, Magnuson JA, Bruinsma BL. 1979. Pulsed nuclear magnetic resonance study of water mobility in flour doughs. Journal of Food Science 44(5):1408-1411.

Leung HK, Magnuson JA, Bruinsma BL. 1983. Water binding of wheat flour doughs and breads as studied by deuteron relaxation. Journal of Food Science 48(1):95-99.

Leung HK, Steinberg MP. 1979. Water binding of food constituents as determined by

NMR, freezing, sorption and dehydration. Journal of Food Science 44:1212

Levin RL. 1979. Water permeabilty of yeast cells at subzero temperatures. Journal of Membrane Biology 46:191-214.

Levine H, Slade L. 1988. Themomechanical properties of small-carbohydrate-water glasses and "rubbers". kinetically metastable systems at sub-zero temperatures. Journal of the Chemical Society, Faraday Transactions 84(8):2619-2633.

Levine H, Slade L. 1991. A food polymer science approach to structure-property relationships in aqueous food systems: nonequilibrium behavior of carbohydrate-water systems. In: Levine H, Slade L. water relationships in foods : advances in the 1980s and trends for the 1990s. New York: Plenum Press. p 29-102

Levitt J. 1962. A sulfhydryl-disulphide hypothesis of frost injury and resistance in plants. Journal of Theoretical Biology 3:355-391.

Lewis JG, Learmonth RP, Attfield PV, Watson K. 1997. Stress co-tolerance and trehalose content in baking strains of Saccharomyces cerevisiae. Journal of Industrial Microbiology and Biotechnology 18(1):30-36.

Lewis JG, Learmonth RP, Watson K. 1993. Role of growth phase and ethanol in freeze-thaw stress resistance of Saccharomyces cerevisiae. Applied and Environmental Microbiology 59(4):1065-1071 .

Liao JC, Lightfoot ENJ, Jolly SO, Jacobson GK. 1988. Application of characteristic reaction paths rate-limiting capability of phosphofructokinase in yeast fermentation. Biotechnology and Bioengineering 31(8):855-868.

Lide DR. 2004. CRC handbook of chemistry and physics. 85th ed. Cleveland, Ohio: CRC Press.

Lim MH, Reid DS. 1991. Studies of reaction kinetics in relation to the Tg' of polymers in frozen model systems. In: Levine H, Slade L, editors. Water relationships in foods : advances in the 1980s and trends for the 1990s. New York: Plenum Press. p 103-122.

Lind I. 1988. Thawing of minced meat and dough: thermal data and mathematical modelling. In: Proceedings from the international symposium on progress in food preservation processes. April 12-14 1988 , Brussels, Belgium. Brussels, Belgium: CERIA. p 101-111.

Lind I. 1991a. Mathematical modelling of the thawing process. Journal of Food Engineering 14(1):1-23.

Lind I. 1991b. The measurement and prediction of thermal properties of food during freezing and thawing - a review with particular reference to meat and dough. Journal of Food Engineering 13(4):285-319.

Lind I, Rask C. 1991. Sorption isotherms of mixed minced meat, dough, and bread crust. Journal of Food Engineering 14(4):303-315.

Lindsay MP, Skerritt JH. 1999. The glutenin macropolymer of wheat flour doughs: structure-function perspectives. Trends in Food Science and Technology 10(8):247-253.

Lindsay MP, Skerritt J.H. 2000. Immunocytochemical localization of gluten proteins uncovers structural organization of glutenin macropolymer. Cereal Chemistry 77(3):360-369.

Lindstrom TR, Slade L, inventors; General Foods Corporation, assignee. 1983 Feb 15. Frozen dough for bakery products. United States Patent 4 374 151.

Ling RS, Hoseney RC. 1977. Effect of certain nutrients on the gas produced in preferments. Cereal Chemistry 54(3):597-604.

Loenner C, Preve-Akesson K. 1988. Acidification properties of lactic acid bacteria in rye sour doughs. Food Microbiology 5(1):43-58.

Lonergan D, Larsen M, Sierzant R, inventors; The Pillsbury Company, assignee. 1998 Sep 8. Ice resistant frozen dough, and method of making. United States Patent 5 804 233.

Lonergan DA, McIntyre TL, inventors; The Pillsbury Company, assignee. 2003 April 3. Frozen dough having decreased proof time. United States Patent Application 20030064138.

Lonergan DA, Pesheck PS, Hahn PW, inventors; The Pillsbury Company, assignee. 1997 Sep 30. Alcohol and polyol-containing doughs and method of making. United States Patent 5 672 369.

Lookhart G, Bean S. 2000. Cereal proteins: composition of their major fractions and methods for identification. In: Kulp K, Ponte JG. Handbook of cereal science and

technology. 2nd ed. New York: Marcel Dekker. p 363-384.

Lorenz K, Kulp K. 1995. Freezing of doughs for the production of breads and rolls in the united states. In: Kulp K, Lorenz K, Brummer JM, editors. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists. p 135-65.

Loureiro-Dias MC, Peinado JM. 1982. Effect of ethanol and other alkanols on the maltose transport- system of Saccharomyces-cerevisiae. Biotechnology Letters 4(11):721-724.

Lovelock JE. 1953. The haemolysis of human red blood-cells by freezing and thawing. Biochimica et Biophysica Acta 10:414-426.

Lovgren T, Hautera P. 1977. Transport and utilisation of maltose by Saccharomyces cerevisiae. Brewers Digest 52:43-47.

Lu W, Grant LA. 1999. Effects of prolonged storage at freezing temperatures on starch and baking quality of frozen doughs. Cereal Chemistry 76(5):656-662.

MacRitchie F. 1976. The liquid phase of dough and its role in baking. Cereal Chemistry 53(3):318-326.

Macritchie F. 1977. Flour lipids and their effects in baking. Journal of the Science of Food and Agriculture 28:53-58.

MacRitchie F. 1978. Differences in baking quality between wheat flours. Journal of Food Technology 13(3):187-194.

MacRitchie F. 1981. Flour lipids: theoretical aspects and functional properties. Cereal Chemistry 58(3):156-158.

MacRitchie F. 1984. Baking quality of wheat flours. Advances in Food Research 29:201-277.

Macritchie F, Gras PW. 1973. The role of flour lipids in baking. Cereal Chemistry 50:292.

Mahoney DH, Foy JJ. 2003. Yeast fermentations. In: Kulp K, Lorenz KJ. handbook of dough fermentations. New York: Marcel Dekker.

Mansure JJC, Panek AD, Crowe LM, Crowe JH. 1994. Trehalose inhibits ethanol

effects on intact yeast cells and liposomes. Biochimica et Biophysica Acta 1191(2):309-316.

Marangoni AG. 2003. Enzyme kinetics : a modern approach. Hoboken, New Jersey: Wiley-Interscience.

Marechal PA, Martinez-DeMaranon I, Molin P, Gervais P. 1995. Yeast cell responses to water potential variations. International Journal of Food Microbiology 28(2):277-287.

Marek CJ, Bushuk W. 1967. Study of gas production and retention in dough with a modified Brabender oven-rise recorder. Cereal Chemistry 44:300-307 .

Marion D, Dubreil L, Wilde PJ, Clark DC. 1998. Lipids, lipid-protein interactions and the quality of baked cereal products. In: Hamer R, Hoseney RC, editors. Interactions: the keys to cereal quality. St. Paul, Minnesota: American Association of Cereal Chemists. p 131-168.

Marston P, Macritchie F. 1985. Lipids: effects on the breadmaking qualities of australian flours. Food Technology in Australia 37(8):362-365.

Martinez-Anaya MA. 1996. Enzymes and bread flavor. Journal of Agricultural and Food Chemistry 44(9):2469-2480.

Martinez-DeMaranon I, Gervais P, Molin P. 1997. Determination of cells' water membrane permeability: unexpected high osmotic permeability of Saccharomyces cerevisiae. Biotechnology and Bioengineering 56(1):62-70.

Masovic S, Jankovic M. 2002. Quality changes of yeasted dough influenced by different freezing regime. In: Chrz V, editor. cryogenics 2002. The seventh cryogenics 2002 IIR international conference proceedings. April 23-26 2002, Praha, Czech RepublicIcaris.

Mauricio JC, Salmon JM. 1992. Apparent loss of sugar-transport activity in Saccharomyces- cerevisiae may mainly account for maximum ethanol-production during alcoholic fermentation. Biotechnology Letters 14(7):577-582.

Mauritzen CM, Stewart PR. 1965. The ultracentrifugation of doughs made from wheat flour. Australian Journal of Biological Science 18:173.

Mazur P. 1966. Physical and chemical basis of injury in single-celled micro-organisms subjected to freezing and thawing. In: Meryman HT, editor. cryobiology. London:

Academic Press. p 214-316.

Mazur P. 1970. Cryobiology: the freezing of biological systems. Science 168:939-949.

Mazur P. 1976. Mechanisms of injury and protection in cells and tissues at low temperatures. Les colloques de l'institut national de la sante et de la recherche medical (cryoimmunology) INSERM 62:37-60.

Mazur P, Schmidt JJ. 1968. Interactions of cooling velocity, temperature, and warming velocity on the survival of frozen and thawed yeast. Cryobiology 5(1):1.

McCormack G, Panozzo J, Bekes F, MacRitchie F. 1991. Contributions to breadmaking of inherent variations in lipid content and composition of wheat cultivars. I. Results of survey. Journal of Cereal Science 13(3):255-261.

Mecham DK. 1971. lipids. In: Pomeranz Y, Hlynka I, editors. Wheat chemistry and technology. 2nd ed. St. Paul, Minnesota: American Association of Cereal Chemists. p 393-452.

Medcalf DG, Gilles KA. 1968. The function of starch in dough. Cereal Science Today 13(10): 382-85, 388, 392.

Medintz I, Jiang H, Han EK, Cui W, Michels CA. 1996. Characterization of the glucose-induced inactivation of maltose permease in Saccharomyces cerevisiae. Journal of Bacteriology 178(8):2245-2254.

Meric L, Lambert-Guilois S, Neyreneuf O, Richard-Molard D. 1995. Cryoresistance of baker's yeast Saccharomyces cerevisiae in frozen dough: contribution of cellular trehalose. Cereal Chemistry 72(6):609-615.

Meuser F, Suckow P. 1986. Non-starch polysaccharides. In: Blanshard JMV, Frazier PJ, Galliard T, editors. Chemistry and physics of baking. materials, processes, and products. p 42-61.

Michaelis L, Menten ML. 1913. Die kinetik der invertinwirkung. Biochemische Zeitschrift 14:333-369.

Monod J. 1949. The growth of bacterial cultures. Annual Review of Microbiology 3:371.

Morrison WR, Law CN, Wylie LJ, Coventry AM, Seekings J. 1989. The effect of group 5 chromosomes on the free polar lipids and breadmaking quality of wheat. Journal of

Cereal Science 9(1):41-51.

Moss R. 1972. A study of the microstructure of bread doughs. Food Research Quarterly 32(3):50-56.

Mwesigye PK, Barford JP. 1996. Batch growth and transport kinetics of utilization of mixtures of sucrose and maltose by Saccharomyces cerevisiae. Journal of Fermentation and Bioengineering 82(2):101-108.

Myers DK, Attfield PV. 1999. Intracellular concentration of exogenous glycerol in Saccharomyces cerevisiae provides for improved leavening of frozen sweet doughs. Food Microbiology 16(1):45-51.

Myers DK, Joseph VM, Pehm S, Galvagno M, Attfield PV. 1998. Loading of Saccharomyces cerevisiae with glycerol leads to enhanced fermentation in sweet bread doughs. Food Microbiology 15(1):51-58.

Myers DK, Lawlor DTM, Attfield PV. 1997. Influence of invertase activity and glycerol synthesis and retention on fermentation of media with a high sugar concentration by Saccharomyces cerevisiae. Applied and Environmental Microbiology 63(1):145-150.

Myhre DV. 1970. The function of carbohydrates in baking. Baker's Digest 44(3):32-35, 38-39, 60.

Nemeth LJ, Paulley FG, Preston KR. 1996. Effects of ingredients and processing conditions on the frozen dough bread quality of a Canada western red spring wheat flour during prolonged storage. Food Research International 29(7):609-616.

Neyreneuf O, Delpuech B. 1993. Freezing experiments on yeasted dough slabs. effects of cryogenic temperatures on the baking performance. Cereal Chemistry 70(1):109-111.

Neyreneuf O, Van der Plaat JB. 1991. Preparation of frozen french bread dough with improved stability. Cereal Chemistry 68(1):60-66.

Oda Y, Uno K, Ohta S. 1986. Selection of yeasts for breadmaking by the frozen-dough method. Applied and Environmental Microbiology 52(4):941-943.

Orlowski JH. 1987. THE kinetics of uptake and metabolism of glucose, fructose and sucrose by S. cerevisiae. [PhD dissertation]. Sydney, Australia: University of Sydney. 485 pages.

Orlowski JH, Barford JP. 1987. The mechanism of uptake of multiple sugars by Saccharomyces-cerevisiae in batch culture under fully aerobic conditions. Applied Microbiology and Biotechnology 25(5):459-463.

Panchal CJ, Stewart GG. 1979. The effect of osmotic pressure on the production and excretion of ethanol and glycerol by a brewing yeast strain. Journal of the Institute of Brewing 86(5):207-210.

Park JI, Grant CM, Attfield P.V., Dawes I.W. 1997. The freeze-thaw stress response of the yeast Saccharomyces cerevisiae is growth phase specific and is controlled by nutritional state via the ras-cyclic amp signal transduction pathway. Applied and Environmental Microbiology 63(10):3818-3824.

Parshotam AA, Bhamidimarri RSM, Wake GC. 1991. A simple method for obtaining good bounds for solutions of reaction-diffusion equations with nonlinear kinetics. Chemical Engineering Science 46(1):49-55.

Pascual C, Alonso A, Garcia I, Romay C, Kotyk A. 1988. Effect of ethanol on glucose transport, key glycolytic enzymes and proton extrusion in Saccharomyces cerevisiae. Biotechnology and Bioengineering 32:374-378.

Peinado JM, Loureiro-Dias MC. 1986. Reversible loss of affinity induced by glucose in the maltose-proton symport of Saccharomyces-cerevisiae. Biochimica et Biophysica Acta 856(2):189-192.

Pereira MD, Eleutherio ECA, Panek AD. 2001. Acquisition of tolerance against oxidative damage in Saccharomyces cerevisiae. BMC Microbiology [serial online] 1(11): available from BioMed Central (www.biomedcentral.com). Posted July 16 2001.

Peringer P, Blachere H, Corrieu G, Lane AG. 1974. A generalised model for the growth kinetics of Saccharomyces cerevisiae with experimental determination of parameters. Biotechnology and Bioengineering 16:431-454.

Pham QT, Mawson RF. 1997. Moisture migration and ice recrystallization in frozen foods. In: Erickson MC, Hung Y-C, editors. quality in frozen food. New York: Chapman & Hall. p 67-91.

Pomeranz Y. 1980. Molecular approach to breadmaking: an update and new perspectives. Baker's Digest 54(1):20-26.

Pomeranz Y. 1988. Wheat: chemistry and technology. 3rd ed. St. Paul, Minnesota: American Association of Cereal Chemists.

Pomeranz Y, Chung OK. 1978. Interaction of lipids with proteins and carbohydrates in breadmaking. Journal of the American Oil Chemists Society 55(2):285-289.

Pomeranz Y, Finney KF, Hoseney RC. 1970. Molecular approach to breadmaking. Baker's Digest 44(6):22-26, 28, 62.

Ponte JGJ. 1973. bread. In: Pyler EJ. Baking science & technology. 2nd ed. Chicago: Siebel Publishing Company. p 675-742.

Potus J, Drapron R, Rakotozafy L. 1992. Role de l'eau sur les interactions moleculaires conduisant a la formation des pates de farines de ble [The role of water in molecular interactions leading to formation of wheat doughs.]. Industries des Cereales 79:13-21.

Potus J, Poiffait A, Drapron R. 1994. Influence of dough-making conditions on the concentration of individual sugars and their utilization during fermentation. Cereal Chemistry 71(5):505-508.

Preston KR. 1998. Protein-carbohydrate interactions. In: Hamer R, Hoseney RC, editors. interactions: the keys to cereal quality. St. Paul, Minnesota: American Association of Cereal Chemists. p 81-94.

Pyler EJ. 1988. Baking science and technology. 3rd ed. Merriam, Kansas, Sosland Publishing Company.

Qiang L, Schmidt RK, Teo B, Karplus PA, Brady JW. 1996. Molecular dynamics studies of the hydration of α-α-trehalose. Journal of the American Chemical Society 119(33):7851-7862.

Rasper VF, Walker CE. 2000. Quality evaluation of cereals and cereal products. In: Kulp K, Ponte JG. Handbook of cereal science and technology. 2nd ed. New York: Marcel Dekker. p 505-38.

Räsänen J. 1998. Prefermented frozen lean wheat doughs. [DTech dissertation]. Espoo, Finland: Helsinki University of Technology. Espoo, Finland. 68 pages.

Räsänen J, Blanshard JMV, Mitchell J.R., Derbyshire W., Autio K. 1998. Properties of frozen wheat doughs at subzero temperatures. Journal of Cereal Science 28(1):1-14.

Räsänen J, Blanshard JMV, Siitari-Kauppi M , Autio K. 1997. Water distribution in frozen lean wheat doughs. Cereal Chemistry 74(6):806-813.

Reed G, Nagodawithana TW. 1991. Yeast technology. 2nd ed. New York: Van Nostrand Reinhold.

Reid DS. 1993. Basic physical phenomena in the freezing and thawing of plant and animal tissues. In: Mallett CP, editor. frozen food technology. p 1-19.

Reid DS. 1998. Freezing - nucleation in foods and antifreeze actions. In: Reid DS, editor. The properties of water in foods. International Symposium on the Properties of Water (ISOPOW) 6. March 2-8 1996, Santa Rosa, California. London: Blackie Academic & Professional. p 275-286 .

Reid DS, Hsu J, Kerr W. 1993. Calorimetry. In: Blanshard JMV, Lillford P, editors. The glassy state in foods. Loughborough, Leicestershire: Nottingham University Press. p 123-132.

Reid DS, Kerr W, Hsu J. 1994. The glass transition in the freezing process. Journal of Food Engineering 22:483-494.

Reid DS. 1997. Overview of physical/chemical aspects of freezing . In: Erickson MC, Hung Y-C, editors. Quality in frozen food. New York: Chapman & Hall. p 10-28.

Reinhardt C, Volker B, Martin HJ, Kneiseler J, Fuhrmann GF. 1997. Different activation energies in glucose uptake in Saccharomyces cerevisiae dfy1 suggest two transport systems. Biochimica Et Biophysica Acta 1325(1):126-134.

Richards AB, Dexter LB. 2001. Trehalose. In: Nabors LO, editor. Alternative sweeteners. 3rd ed. New York: Marcel Dekker. p 423-61.

Robinow CF, Johnson BF. 1991. Yeast cytology: an overview. In: Rose AH, Harrison JH, editors. the yeasts. 2nd ed. Volume 4. London: Academic Press. p 7-120.

Roels JA, Kossen NWF. 1978. On the modelling of microbial metabolism. Progress in industrial microbiology 14:95-203.

Rogers D, Langemeier J. 1995. Sugars in doughs and resultant breads and biscuits. Technical Bulletin, American Institute of Baking Research Department 17(5):1-5.

Roman-Gutierrez AD, Guilbert S, Cuq B. 2002a. Distribution of water between wheat

flour components: a dynamic water vapour adsorption study. Journal of Cereal Science 36:347-355.

Roman-Gutierrez AD, Guilbert S, Cuq B. 2002b. Frozen and unfrozen water contents of wheat flours and their components. Cereal Chemistry 79(4):471-475.

Roman W, editor. 1957. Yeasts. The Hague: Dr. W. Junk Publishers.

Roos YH, Karel M, Kokini JL. 1996. Glass transitions in low moisture and frozen foods: effects on shelf life and quality. Food Technology 50(11):95-108.

Rouzaud O, Martinez-Anaya MA. 1993. Effect of processing conditions on oligosaccharide profile of wheat sourdoughs. Zeitschrift fur Lebensmittel Untersuchung und Forschung 197(5):434-439.

Ruan RR, Chen PL. 1998. Water in foods and biological materials : a nuclear magnetic resonance approach. Lancaster, Pennsylvania: Technomic Publishing Company.

Ruan RR, Wang XA, Chen PL, Fulcher RG, Pesheck P, Chakrabarti S. 1999. Study of water in dough using nuclear magnetic resonance. Cereal Chemistry 72(2):231-235.

Ruckold S, Isengard HD, Hanss J, Grobecker KH. 2003. The energy of interaction between water and surfaces of biological reference materials. Food Chemistry 82(1):51-59.

Sa MM, Sereno AM. 1994. Glass transitions and state diagrams for typical natural fruits and vegetables. Thermochimica Acta 246:284-297.

Saalfeld U, Freund W. 1998. Quantitative determination of ethanol as a method to investigate fermenting activity in yeast leavened wheat doughs [Quantitative Ethanolbestimmung als Gaerpruefverfahren hefeglockerter Weizenteige]. Deutsche Lebensmittel Rundschau 94(9):292-296.

Sablani SS, Baik OD, Marcotte M. 2002. Neural networks for predicting thermal conductivity of bakery products. Journal of Food Engineering 52(3):299-304.

Saff EB, Nagle RK. 1996. Fundamentals of differential equations. 4th ed. Reading, Massachusetts: Addison-Wesley Publishing Company.

Sakurai M, Kawai H, Inoue Y, Hino A, Kobayashi S. 1995. Effects of trehalose on the water structure in yeast cells as studied by in vivo H-1 NMR spectroscopy. Bulletin of

the Chemical Society of Japan 68(12):3621-3627.

Sakurai M, Murata M, Inoue Y, Hino A, Kobayashi S. 1997. Molecular-dynamics study of aqueous solution of trehalose and maltose: implication for the biological function of trehalose. Bulletin of the Chemical Society of Japan 70(4):847-858.

Salgueiro SP, Sacorreia I, Novais JM. 1988. Ethanol-induced leakage in Saccharomyces-cerevisiae - kinetics and relationship to yeast ethanol tolerance and alcohol fermentation productivity. Applied and Environmental Microbiology 54(4):903-909.

Salmon JM, Vincent O, Mauricio JC, Bely M, Barre P. 1993. Sugar-transport inhibition and apparent loss of activity in Saccharomyces-cerevisiae as a major limiting factor of enological fermentations. American Journal of Enology and Viticulture 44(1):56-64.

Sandstedt RM. 1961. the function of starch in the baking of bread . Baker's Digest 35(3):36.

Sandstedt RM, Schaumburg, Lorene, Fleming J. 1954. The microscopic structure of bread and dough. Cereal Chemistry 31:43-49.

Sano F, Asakawa N, Inoue Y, Sakurai M. 1999. A dual role for intracellular trehalose in the resistance of yeast cells to water stress. Cryobiology 39(1):80-87.

Schultz A. 1965. Methodische untersuchungen zuer ermittlung der Triebkraft von Backhefen. Brot und Gebaeck 19(4):61-65.

Schwartz GJ, Diller KR. 1983. Osmotic response of individual cells during freezing. I. Experimental volume measurements. Cryobiology 20:61-77.

Schwartzberg HG, Hartel RW, editors. 1992. Physical chemistry of foods. New York: Marcel Dekker.

Seguchi M, Nikaidoo S, Morimoto N. 2003. Centrifuged liquid and breadmaking properties of frozen-and- thawed bread dough. Cereal Chemistry 80(3):264-268.

Seppi A. 1984. Biochemistry of sugars in fermentation of bread dough. Rivista della Societa Italiana di Scienza Dell'Alimentazione 13(4):313-320.

Serrano R, De la Fuente G. 1974. Regulatory properties of the constitutive hexose transport in Saccharomyces cerevisiae. Molecular and Cellular Biochemistry 5:161.

Shah P, Campbell GM, McKee SL, Rielly CD. 1998. Proving of bread dough: modelling the growth of individual bubbles. Food and Bioproducts Processing 76(C2):73-79.

Shannon E. 2003. An investigation of the carbon dioxide production rates in bread dough after frozen storage [BTech dissertation]. Palmerston North, New Zealand: Massey University. 47 pages.

Sharadanant R, Khan K. 2003. Effect of hydrophilic gums on frozen dough. I. Dough quality. Cereal Chemistry 80(6):764-772.

Shelton DR, D'Appolonia BL. 1985. Carbohydrate functionality in the baking process. Cereal Foods World 30(7):437-442.

Shelton DR, Lee WJ. 2000. Cereal carbohydrates. In: Kulp K, Ponte JG. Handbook of cereal science and technology . 2nd ed. New York: Marcel Dekker. p 385-416.

Shogren MD, Finney K.F., Hoseney R.C. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. I. Solubilizing gluten and flour protein. Cereal Chemistry 46(2):93-102.

Shogren MD, Finney KF, Rubenthaler GL. 1977. Note on determination of gas production. Cereal Chemistry 54(3):665-668.

Shogren MD, Hashimoto S, Pomeranz Y. 1987. Cereal pentosans: their estimation and significance. II. Pentosans and breadmaking characteristics of hard red winter wheat flours. Cereal Chemistry 64(1):35-38.

Simatos D, Blond G. 1993. Some aspects of the glass transition in frozen foods systems. In: Blanshard JMV, Lillford P, editors. The glassy state in foods. Loughborough, Leicestershire: Nottingham University Press. p 395-416.

Siro M-R, Loevgren T. 1979. On the maltose metabolism of yeast. Baker's Digest 53(4):24-29.

Skovholt O, Bailey CH. 1935. Free and bound water in bread doughs. Cereal Chemistry 12:321-355.

Smogrovicova D, Domeny Z, Svitel J. 2001. Modeling of saccharide utilization in primary beer fermentation with yeasts immobilized in calcium alginate. Applied Biochemistry and Biotechnology 94(2):147-158.

Sonnleitner B, Kapelli O. 1986. Growth of Saccharomyces cerevisiae is controlled by its limited respiratory capacity: formulation and verification of a hypothesis. Biotechnology and Bioengineering 28:927-937.

Stambuk BU, Batista AS, De Araujo PS. 2000. Kinetics of active sucrose transport in Saccharomyces cerevisiae. Journal of Bioscience and Bioengineering 89(2):212-214.

Stauffer CE. 1993. frozEn bakery products. In: Mallett CP, editor. Frozen food technology. p 303-332.

Stecchini ML, Maltini E, Venir E, Del Torre M, Prospero L. 2002. Properties of wheat dough at sub-zero temperatures and freeze tolerance of a baker's yeast (Saccharomyces cerevisiae). Journal of Food Science 67(6):2196-2201.

Sugihara TF, Kline L. 1968. Factors affecting the stability of frozen bread doughs. II - Prepared by the sponge and dough method. Baker's Digest 42(5):51-53, 56, 59.

Suomalainen H, Dettwiler J, Sinda E. 1972. Alpha-glucosidase and leavening of baker's yeast. Process Biochemistry 7(5):16-19, 22.

Sweere APJ, Giesselbach J, Barendse R, De Krieger R, Honderd G, Luyben K. 1988. Modelling the dynamic behavior of Saccharomyces cerevisiae and its application in control experiments. Applied Microbiology and Biotechnology 28(2):116-127.

Tanaka Y, Kawaguchi M, Miyatake M. 1976. Studies on the injury of yeast in frozen dough. II. Effect of ethanol on the frozen storage of baker's yeast [Japanese]. Journal of Food Science and Technology [Nippon Shokuhin Kogyo Gakkaishi] 23(9):419-424.

Tanaka Y, Kawakami K, Takano H. 1980. Effect of prestorage on freeze-tolerance of compressed bakers yeast in frozen dough [Japanese]. Report of National Food Research Institute [Shokuryo Kenkyusho Kenkyu Hokoku] 36:64-71.

Tanaka Y, Miyatake M. 1975. Studies on the injury of yeast in frozen dough. I. Effect of pre-fermentation before freezing on the injury of yeast. Journal of Food Science and Technology [Nippon Shokuhin Kogyo Gakkaishi] 22(8):366-371.

Tang RT, Robinson RJ, Hurley WC. 1972. Quantitative changes in various sugar concentrations during breadmaking. Baker's Digest 46(4):48-55.

Tanghe A, Van Dijck P, Colavizza D, Thevelein JM. 2004. Aquaporin-mediated improvement of freeze tolerance of Saccharomyces cerevisiae is restricted to rapid

freezing conditions. Applied and Environmental Microbiology 70(6):3377-3382.

Tanghe A, Van Dijck P, Dumortier F, Teunissen A, Hohmann S, Thevelein JM. 2002. Aquaporin expression correlates with freeze tolerance in baker's yeast, and overexpression improves freeze tolerance in industrial strains. Applied and Environmental Microbiology 68(12):5981-5989.

Thierie J. 2004. Modeling threshold phenomena, metabolic pathways, switches and signals in chemostat-cultivated cells: the Crabtree effect in Saccharomyces cerevisiae. Journal of Theoretical Biology 226(4):483-501.

Thorn JA, Ross JW. 1960. Determination of yeast growth in doughs. Cereal Chemistry 37:415.

Toledo R, Steinberg MP, Nelson AI. 1968. Quantitative determination of bound water by NMR. Journal of Food Science 33(3):315-317.

Tolstoguzov V. 1997. Thermodynamic aspects of dough formation and functionality. Food Hydrocolloids 11(2):181-193.

Tomas SA, Harren FJM. 2001. Kinetics of ethanol and acetaldehyde production in fermenting wheat dough by laser-based trace gas detection. Food Science and Technology International 7(4):307-315.

Udani KH, Nelson AI, Steinberg MP. 1969. Rate of moisture adsorption by wheat flour and its relation to physical, chemical and baking characteristics. Food Technology 22(12):1561-1564.

Ugalde TD, Jenner CF. 1990. Substrate gradients and regional patterns of dry matter decomposition within developing wheat endosperm. I. Carbohydrates. Australian Journal of Plant Physiology 17:377-394.

Vackier MC, Hills BP, Rutledge DN. 1999. An NMR relaxation study of the state of water in gelatin gels. Journal of Magnetic Resonance 138(1):36-42.

Vail GE, Bailey CH. 1940. The state of water in colloidal gels: free and bound water in bread doughs. Cereal Chemistry 17:397.

Van den Berg L. 1959. The effect of addition of sodium and potasssium chloride to the reciprocal system: KH_2PO_4-Na_2HPO_4-H_2O on pH and composition during freezing. Archives of Biochemistry and Biophysics 84: 305-315.

Van den Berg L. 1968. Physicochemical changes in foods during freezing and subsequent storage. In: Hawthorn J, Rolfe EJ. Low temperature biology of foodstuffs. Oxford: Pergamon Press. p 205-19.

Van der Plaat JB. 1988. Baker's yeast in frozen dough - state of the art. In: Asp N-G, editor. Cereal science and technology in Sweden. Lund, Sweden: University of Lund. p 110-129.

Van Dijck P., Colavizza D, Smet P, Thevelein JM. 1995. Differential importance of trehalose in stress resistance in fermenting and nonfermenting Saccharomyces cerevisiae cells. Applied and Environmental Microbiology 61(1):109-115.

Van Hoek P, De Hulster E, Van Dijken JP, Pronk JT. 2000. Fermentative capacity in high-cell-density fed-batch cultures of baker's yeast. Biotechnology and Bioengineering 68(5):517-523.

Varo P, Westermarck-Rosendahl C, Hyvonen L, Koivistoinen P. 1979. Baking behavior of different sugars and sugar alcohols as determined by high-pressure liquid-chromatography. Lebensmittel Wissenschaft und Technologie 12(3):153-156.

Varriano-Marston E. 1995 . Packaging materials for frozen and refrigerated doughs. In: Kulp K, Lorenz K, Brummer JM. Frozen and refrigerated doughs and batters. St. Paul, Minnesota: American Association of Cereal Chemists. p 245-54.

Varriano-Marston E, Hsu KH, Mahdi J. 1980. Rheological and structural changes in frozen dough. Baker's Digest 54(1):32-34, 41.

Verstrepen KJ, Iserentant D, Malcorps P, Derdelinckx G, Van Dijck P, Winderickx J, Pretorius IS, Thevelein JM, Delvaux FR. 2004. Glucose and sucrose: hazardous fast-food for industrial yeast? Trends in Biotechnology 22(10):531-537.

Vivier D, Rivemale M, Reverbel JP, Ratomahenina R, Galzy P. 1994. Study of the growth of yeasts from feta cheese. International Journal of Food Microbiology 22(2-3):207-215.

Waley SG. 1981. An easy method for the determination of initial rates. Biochemical Journal 183:1009-1012.

Walker GM. 1998. Yeast physiology and biotechnology. Chichester: J. Wiley & Sons.

Walstra P. 1996. Dispersed systems: basic considerations. In: Fennema OR, editor.

Food Chemistry. 3rd ed. New York: Marcel Dekker. p 95-156.

Wang D, Xu Y, Hu J, Zhao G. 2004. Fermentation kinetics of different sugars by apple wine yeast Saccharomyces cerevisiae. Journal of the Institute of Brewing 110(4):340-346.

Wang M, Hamer RJ, Van Vliet T, Oudgenoeg G. 2002. Interaction of water extractable pentosans with gluten protein: effect on dough properties and gluten quality. Journal of Cereal Science 36:22-37.

Webb T, Heaps PW, Eggitt PWR, Coppock JBM. 1970. A rheological investigation of the role of water in wheat flour doughs. Journal of Food Technology 5:65.

Weegels PL, Van de Pijpekamp AM, Graveland A, Hamer RJ, Schofield JD. 1996. Depolymerisation and re-polymerisation of wheat glutenin during dough processing. I. Relationships between glutenin macropolymer content and quality parameters. Journal of Cereal Science 23(2):103-111.

Weusthuis RA, Adams H, Scheffers WA, Van Dijken JP. 1993. Energetics and kinetics of maltose transport in Saccharomyces-cerevisiae - a continuous-culture study. Applied and Environmental Microbiology 59(9):3102-3109.

White GW, Cakebread SH. 1966. The glassy state in certain sugar-containing food products. Journal of Food Technology 1:73-82.

Wilbrandt W, Rosenberg T. 1961. The concept of carrier transport and its corrolaries. Pharmacological Reviews 13:109-183.

Wolt MJ, D'Appolonia BL. 1984a. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chemistry 61(3):209-212.

Wolt MJ, D'Appolonia BL. 1984b. Factors involved in the stability of frozen dough. II. the effects of yeast type, flour type, and dough additives on frozen-dough stability. Cereal Chemistry 61(3):213-221.

Wong M. 1997. Modelling of a direct osmotic concentration membrane system. [PhD dissertation]. Palmerston North, New Zealand: Massey University. 292 pages.

Wood PS, Daniels N.W.R., Greenshields RN. 1972. The effect of water on lipid binding in doughs mixed to low work levels. Journal of Food Technology 7(2):183-189.

Wrede C, Volker B, Kuntzel H, Fuhrmann GF. 1992. Kinetic analysis of glucose transport in wild-type and transporter-deficient Saccharomyces cerevisiae strains under glucose repression and derepression. Journal of Biotechnology 27(1):47-57.

Xie G, Timasheff SN. 1997. The thermodynamic mechanism of protein stabilization by trehalose. Biophysical Chemistry 64(1-3):25-43.

Zounis S, Quail KJ, Wootton M, Dickson MR. 2002a. Studying frozen dough structure using low-temperature scanning electron microscopy. Journal of Cereal Science 35(2):135-147.

Zounis S, Quali KJ, Wootton M, Dickson MR. 2002b. Effect of final dough temperature on the microstructure of frozen bread dough. Journal of Cereal Science 36:135-146.

APPENDICES

A1. Yeast metabolite recovery from spiked doughs

Sugar recovery in extractions without heating

Yeast metabolites were extracted by freezing dough in liquid nitrogen, grinding the dough to a powder, homogenising the powder in water and filtering the homogenate. Extracts were analysed with enzymatic assays.

Table A1.1. Glucose recovery from spiked doughs.

code	extraction	assay	total glucose	control-adjusted glucose	added glucose	recovery
			mmol.(100g dough)$^{-1}$			%
G5	A	1	2.77	2.60	2.88	90.4
G5	A	2	2.61	2.45	2.88	84.9
G5	B	1	2.79	2.62	2.88	91.1
G5	B	2	2.65	2.48	2.88	86.2
G5	C	1	2.74	2.57	2.88	89.3
G5	C	2	2.70	2.53	2.88	87.9
G10	A	1	5.52	5.35	5.76	92.9
G10	A	2	5.23	5.06	5.76	87.8
G10	B	1	6.12	5.95	5.76	103.3
G10	B	2	5.92	5.75	5.76	99.7
G10	C	1	5.90	5.73	5.76	99.5
G10	C	2	5.69	5.52	5.76	95.7

Unyeasted doughs made with 5 or 10 g.kg^{-1} added glucose were extracted without heating. Total glucose concentration in dough was adjusted for endogenous glucose by subtracting glucose concentration in unspiked control doughs. Percentage recovery was calculated by dividing 'control adjusted glucose' by 'added glucose' and multiplying by 100.

Table A1.2. Fructose recovery from spiked doughs.

code	extraction	assay	total fructose	control-adjusted fructose	added fructose	recovery
			mmol.(100g dough)$^{-1}$			%
F5	A	1	3.14	2.72	2.88	94.3
F5	A	2	3.40	2.98	2.88	103.2
F5	B	1	3.35	2.94	2.88	101.9
F5	B	2	3.19	2.78	2.88	96.4
F5	C	1	3.30	2.89	2.88	100.2
F5	C	2	3.10	2.69	2.88	93.3
F10	A	1	6.38	5.96	5.72	104.2
F10	A	2	6.11	5.70	5.72	99.6
F10	B	1	6.32	5.91	5.72	103.2
F10	B	2	6.22	5.81	5.72	101.5
F10	C	1	6.51	6.09	5.72	106.5
F10	C	2	6.25	5.83	5.72	101.9

Unyeasted doughs were made with 5 or 10 g.kg^{-1} added fructose and extracted without heating. Calculations as in Table A1.1.

Table A1.3. Sucrose recovery from spiked doughs.

code	extraction	assay	total sucrose	control-adjusted sucrose	added sucrose	recovery
			mmol.(100g dough)$^{-1}$			%
S5	A	1	3.64	1.72	1.52	112.9
S5	A	2	3.26	1.34	1.52	88.3
S5	B	1	3.60	1.68	1.52	110.4
S5	B	2	3.48	1.56	1.52	102.4
S5	C	1	3.68	1.77	1.52	116.0
S5	C	2	3.49	1.57	1.52	103.4
S10	A	1	5.09	3.17	3.02	105.1
S10	A	2	5.02	3.10	3.02	102.7
S10	B	1	5.37	3.46	3.02	114.6
S10	B	2	5.07	3.15	3.02	104.4
S10	C	1	5.16	3.24	3.02	107.5
S10	C	2	5.27	3.36	3.02	111.2

Unyeasted doughs were made with 5 or 10 g.kg^{-1} added sucrose and extracted without heating. Calculations as in Table A1.1.

Table A1.4. Endogenous sugars in control doughs.

extraction	assay	glucose	fructose	sucrose
		mmol.(100g dough)$^{-1}$		
A	1	0.17	0.39	1.94
B	1	0.17	0.43	1.83
C	1	0.18	0.42	1.98
A	2	0.17	0.39	1.94
B	2	0.17	0.43	1.83
C	2	0.18	0.42	1.84
A	3	0.18	0.45	1.97
B	3	0.16	0.42	1.93
C	3	0.16	0.43	1.94
A	4	0.18	0.45	1.97
B	4	0.16	0.42	1.93
C	4	0.16	0.43	

Unyeasted doughs without added sugar were extracted without heating.

Table A1.5. Maltose recovery from spiked doughs.

CODE	extraction	assay	total glucose	control adjusted glucose	total maltose	added maltose	recovery
			mmol/100g				%
M5	A	1	9.46	3.05	1.52	1.44	105.4
M5	A	2	8.71	2.29	1.15	1.44	79.3
M5	B	1	9.23	2.81	1.41	1.44	97.4
M5	B	2	8.43	2.01	1.01	1.44	69.7
M10	A	1	8.64	2.23	1.11	2.86	38.9
M10	A	2	7.81	1.39	0.70	2.86	24.4
M10	B	1	11.31	4.89	2.45	2.86	85.4
M10	B	2	10.27	3.85	1.93	2.86	67.3
control	A	1	5.81				
control	A	2	5.62				
control	B	1	7.51				
control	B	2	6.72				

Unyeasted doughs were made with 5 or 10 $g.kg^{-1}$ added maltose and extracted without heating. Unyeasted doughs spiked with known quantities of maltose ('added maltose') as well as unspiked control doughs were extracted without heating and assayed for maltose. Extracts were treated with α-glucosidase in the assay, which hydrolysed added maltose as well as endogenous maltose and sucrose.

The total glucose from hydrolysed disaccharides plus endogenous glucose was measured in spiked and control doughs. Spiked dough results were corrected for endogenous sugars by subtracting control dough results, which left glucose originating from added maltose ('control-adjusted glucose').

Maltose concentrations were calculated by dividing control-adjusted glucose concentrations by the stoichiometric coefficient of 2. Percentage recovery was calculated by dividing control adjusted maltose by added maltose and multiplying by 100.

Table A1.6. Ethanol recovery from spiked doughs.

code	grind	extraction	assay	EtOH recovered	EtOH added	recovery
				mmol.(100g dough)$^{-1}$		%
E2	1	A	1	3.34	3.44	97.1
E2	1	A	2	3.31	3.44	96.2
E2	1	B	1	3.49	3.44	101.4
E2	1	B	2	3.60	3.44	104.9
E2	1	C	1	3.60	3.44	104.8
E2	1	C	2	3.50	3.44	101.9
E5	1	A	1	8.92	8.57	104.1
E5	1	A	2	8.88	8.57	103.7
E5	1	B	1	10.22	8.57	119.3
E5	1	B	2	10.14	8.57	118.3
E5	1	C	1	9.17	8.57	107.0
E5	1	C	2	9.17	8.57	107.0
E10	1	A	1	17.92	17.07	105.0
E10	1	A	2	18.14	17.07	106.3
E10	1	B	1	14.12	17.07	82.7
E10	1	B	2	13.92	17.07	81.6
E10	1	C	1	19.39	17.07	113.6
E10	1	C	2	19.51	17.07	114.3
E10	1	A	1	16.85	17.07	98.7
E10	1	A	2	16.80	17.07	98.4
E10	1	B	1	16.86	17.07	98.8
E10	1	B	2	16.69	17.07	97.8
E10	2	A	1	15.99	17.07	93.7
E10	2	A	2	16.02	17.07	93.9
E10	2	B	1	16.65	17.07	97.5
E10	2	B	2	16.80	17.07	98.4
E25	1	A	1	40.43	42.15	95.9
E25	1	A	2	39.76	42.15	94.3
E25	1	B	1	37.19	42.15	88.2
E25	1	B	2	37.73	42.15	89.5
E25	2	A	1	39.90	42.15	94.7
E25	2	A	2	39.45	42.15	93.6
E25	2	B	1	40.33	42.15	95.7
E25	2	B	2	39.09	42.15	92.8

Ethanol was added to unyeasted doughs without added sugar at 2, 5, 10 or 25 millilitres per kilogram of dough ('EtOH added'). Doughs were extracted and analysed for ethanol ('EtOH recovered') and recovery calculated as 100 times the ratio of 'EtOH recovered' to 'EtOH added'.

Sugar recovery in extractions with heating

Yeast metabolites were extracted by freezing dough in liquid nitrogen, grinding the dough to a powder, homogenising the powder in water and filtering the homogenate. Extracts were heated at 85˚C for 1h prior to analysis with enzymatic assays.

Table A1.7. Glucose recovery from spiked doughs extracted with heating.

code	extraction	assay	total glucose	control-adjusted glucose	added glucose	recovery
			mmol.(100g dough)$^{-1}$			%
G5H	A	1	3.04	2.78	2.88	96.7
G5H	A	2	2.90	2.64	2.88	91.8
G5H	B	1	2.81	2.56	2.88	88.8
G5H	B	2	2.87	2.62	2.88	90.9
G10H	A	1	5.66	5.40	5.76	93.8
G10H	A	2	5.83	5.57	5.76	96.7
G10H	B	1	5.88	5.62	5.76	97.5
G10H	B	2	5.83	5.57	5.76	96.7

Unyeasted doughs were made with 5 or 10 g.kg^{-1} added glucose and extracted with heating at 85˚C for 1 h. Calculations as in Table A1.1.

Table A1.8. Fructose recovery from spiked doughs extracted with heating.

sample	extraction	assay	total fructose	control-adjusted fructose	added fructose	recovery
			mmol.(100g dough)$^{-1}$			%
F5H	A	1	3.20	3.07	2.88	106.6
F5H	A	2	3.29	3.17	2.88	109.8
F5H	B	1	3.69	3.56	2.88	123.7
F5H	B	2	3.54	3.42	2.88	118.5
F10H	A	1	6.55	6.42	5.72	112.2
F10H	A	2	6.13	6.00	5.72	104.9
F10H	B	1	6.42	6.29	5.72	109.9
F10H	B	2	6.78	6.65	5.72	116.2

Unyeasted doughs were made with 5 or 10 g.kg^{-1} added fructose and extracted with heating at 85˚C for 1 h. Calculations as in Table A1.1.

Table A1.9. Sucrose recovery from spiked doughs extracted with heating.

sample	extraction	assay	total sucrose	control-adjusted sucrose	added sucrose	recovery
			mmol.(100g dough)$^{-1}$			%
S5H	A	1	3.49	1.53	1.52	100.3
S5H	A	2	3.24	1.27	1.52	83.7
S5H	B	1	3.45	1.48	1.52	97.2
S5H	B	2	3.61	1.65	1.52	108.0
S10H	A	1	4.84	2.87	3.02	95.2
S10H	A	2	4.38	2.42	3.02	80.1
S10H	B	1	4.78	2.81	3.02	93.3
S10H	B	2	5.47	3.50	3.02	116.1

Unyeasted were made with 5 or 10 g.kg^{-1} added sucrose and extracted with heating at 85˚C for 1 h. Calculations as in Table A1.1.

Table A1.10. Endogenous sugars in control doughs.

extraction	assay	glucose	fructose	sucrose
		mmol.(100g dough)$^{-1}$		
A	1	0.25	0.27	1.73
A	2	0.27	0.13	2.00
B	1	0.24	0.13	1.94
B	2	0.24	0.03	1.96
C	1	0.27	0.10	2.18
C	2	0.26	0.08	1.98

Unyeasted doughs without added sugar were extracted with heating at 85˚C for 1h.

Table A1.11. Maltose recovery from spiked doughs extracted with heating.

CODE	extraction	assay	total glucose	control adjusted glucose	total maltose	added maltose	recovery
			mmol/100g				%
M5H	A	1	10.30	3.54	1.77	1.44	122.6
M5H	A	2	9.60	2.84	1.42	1.44	98.2
M5H	B	1	9.68	2.92	1.46	1.44	101.1
M5H	B	2	8.90	2.14	1.07	1.44	74.1
M10H	A	1	12.18	5.42	2.71	2.86	94.6
M10H	A	2	11.47	4.71	2.36	2.86	82.3
M10H	B	1	12.17	5.42	2.71	2.86	94.6
M10H	B	2	10.75	4.00	2.00	2.86	69.8
control	A	1					
control	A	2					
control	B	1					
control	B	2					

Unyeasted doughs were made with 5 or 10 $g.kg^{-1}$ added maltose and extracted with heating. Calculations as in Table A1.5.

A2. Sample stability experiment data and ANOVA

Table A2.1. Effect of storage time at room temperature on glucose concentration in heated and unheated extracts of yeasted dough made with 2% sucrose.

	Glucose concentration (mmol.L^{-1})							
fermentation time[A] (min)	0		60		120		180	
heated[B]	no	yes	no	yes	no	yes	no	yes
storage time (hours)[C]								
[D]0.00	6.54		4.31		0.96		0.35	
0.00	6.55		4.18		1.00		0.33	
1.23	6.99	6.50	4.39	4.04	1.41	0.90	0.88	0.27
1.23	7.08	6.58	4.59	4.01	1.76	0.92	1.21	0.26
2.18	7.27	6.48	4.97	3.92	2.34	0.89	1.77	0.25
2.18	7.39	6.40	5.42	3.85	2.85	0.88	2.41	0.27
4.23	7.05	6.43	4.33	3.97	1.40	0.87	0.85	0.26
4.23	6.94	6.40	4.38	3.75	1.68	0.84	1.18	0.24
7.22	7.13	5.90	4.65	3.71	2.20	0.84	1.58	0.24
7.22	7.35	6.13	5.28	3.72	2.91	0.85	2.30	0.25

A. doughs were extracted after fermentation at 30˚C for 0-180 minutes

B. heat treatment was 85˚C for 1h.

C. extracts stored at room temperature in analyser tubes

D. '0.00' unheated results not included in ANOVA

Minitab Analysis of Variance

```
Factor      Type   Levels  Values
store_time  fixed       4  1.23, 2.18, 4.23, 7.22
ferm_time   fixed       4  0, 60, 120, 180
heated      fixed       2  n, y
```

```
Analysis of Variance for glucose, using Adjusted SS for Tests

Source                        DF   Seq SS   Adj SS   Adj MS        F      P
store_time                     3    1.776    1.776    0.592    12.90  0.000
ferm_time                      3  335.116  335.116  111.705  2434.33  0.000
heated                         1   16.933   16.933   16.933   369.02  0.000
store_time*ferm_time           9    0.719    0.719    0.080     1.74  0.120
store_time*heated              3    2.751    2.751    0.917    19.98  0.000
ferm_time*heated               3    0.683    0.683    0.228     4.96  0.006
store_time*ferm_time*heated    9    0.237    0.237    0.026     0.57  0.808
Error                         32    1.468    1.468    0.046
Total                         63  359.683
```

Moisture content of flour and yeast

Table A2.2. Moisture content of flour.

batch[A]	replicate	% moisture
1	A	12.68
1	B	12.59
1	C	12.80
2	A	12.04
2	B	12.26
2	C	11.96
3	A	11.25
3	B	10.84
3	C	11.29

A. Batch 1 was packed on 22/8/03, batch 2 on 6/5/04 and batch 3 on 5/8/04.

Table A2.3. Moisture content of compressed baker's yeast.

replicate	% moisture
1	32.76
2	32.73
3	32.52

Endogenous sugars in flour

Table A2.4. Endogenous sugars in flour, extracted without heating.

batch	extraction	assay	glucose	fructose	sucrose	maltose
			mmol.(100g flour)$^{-1}$			
1	A	1	0.37	0.18	2.33	3.95
1	A	2	0.37	0.09	2.33	3.40
1	B	1	0.17	0.23	2.54	3.50
1	B	2	0.34	0.07	2.69	2.95
1	C	1	0.34	0.30	2.72	3.97
1	C	2	0.38	0.23	2.70	3.28
2	A	1	0.26	0.03	3.04	4.09
2	A	2	0.26	nd[A]	2.96	3.28
2	B	1	0.26	nd	2.81	3.34
2	B	2	0.25	nd	2.96	3.64
2	C	1	0.24	nd	3.09	3.88
2	C	2	0.18	nd	3.32	3.41
3	A	1	0.00	0.14	2.90	3.84
3	A	2	0.11	0.09	2.77	3.01
3	B	1	0.24	nd	3.07	4.45
3	B	2	0.24	nd	2.99	3.97
3	C	1	0.24	nd	2.69	3.71
3	C	2	0.22	nd	2.89	3.76

A. not detected

Table A2.5. Endogenous sugars in flour, extracts heated at 85˚C for 1h.

batch	extraction	assay	glucose	fructose	sucrose	maltose
			mmol.(100g flour)$^{-1}$			
1	A	1	0.33	0.16	2.10	2.93
1	A	2	0.32	0.14	2.40	2.81
1	B	1	0.32	0.14	2.48	2.64
1	B	2	0.31	0.11	2.20	2.84
1	C	1	0.36	0.27	2.80	3.29
1	C	2	0.32	0.19	2.59	3.48
2	A	1	0.21	nd[A]	3.32	2.97
2	A	2	0.17	0.01	2.92	2.48
2	B	1	0.13	0.06	2.76	3.46
2	B	2	0.13	0.02	2.96	3.16
2	C	1	0.22	nd	2.88	3.22
2	C	2	0.21	nd	2.87	3.08
3	A	1	0.18	nd	2.71	2.89
3	A	2	0.12	nd	2.77	3.60
3	B	1	0.20	nd	2.83	3.75
3	B	2	0.1979	nd	2.8296	3.245
3	C	1	0.2541	nd	2.9950	3.259
3	C	2	0.1661	nd	2.9657	3.122

A. not detected

A3. Data from gassing power experiments

Table A3.1. Gassing power of pre-fermented doughs frozen, stored and thawed.

	mean volume (ml)[A]											
Frozen storage time[B]	Fresh[C]		0 hours[D]		3 hours		6 hours		24 hours		48 hours	
Risograph replicate	1	2	1	2	1	2	1	2	1	2	1	2
dough replicate	UNFERMENTED											
1	87.14	88.63	69.58	82.07	75.92	68.84	70.50	85.90	73.49	75.94	-	-
2	77.50	79.55	63.40	61.69	63.95	63.00	50.90	59.66	57.00	55.46	42.18	46.21
3	62.10	61.84	-	-	53.78	60.30	61.64	55.13	34.88	-	53.42	51.83
	30 MIN PREFERMENTED[E]											
1	90.03	90.06	70.92	78.47	71.54	74.22	71.38	72.22	-	-	-	-
2	81.17	86.21	91.08	70.92	74.46	72.72	73.53	70.81	60.47	60.43	59.53	52.47
3	94.48	97.12	38.59	73.81	67.34	73.02	73.56	48.71	39.58	63.40	59.65	62.41
	60 MIN PREFERMENTED											
1	76.74	91.08	70.98	63.41	71.92	70.77	61.73	62.99	-	-	-	-
2	91.17	58.19	78.93	82.68	70.30	75.72	62.47	65.10	51.38	52.00	45.79	45.78
3	89.43	93.36	78.93	82.68	63.33	68.95	60.16	64.50	45.20	42.37	41.10	32.73
	90 MIN PREFERMENTED											
1	83.68	89.35	76.60	77.70	65.82	71.54	44.78	41.28	-	-	-	-
2	82.46	89.43	70.70	56.83	54.67	60.45	48.84	47.98	36.00	-	29.96	28.91
3	80.87	90.37	62.47	63.72	58.16	64.02	-	-	33.13	58.35	27.14	-

A. Area under the cumulative gas volume curve divided by the time of data collection

B. doughs frozen at -15˚C and stored at -20˚C

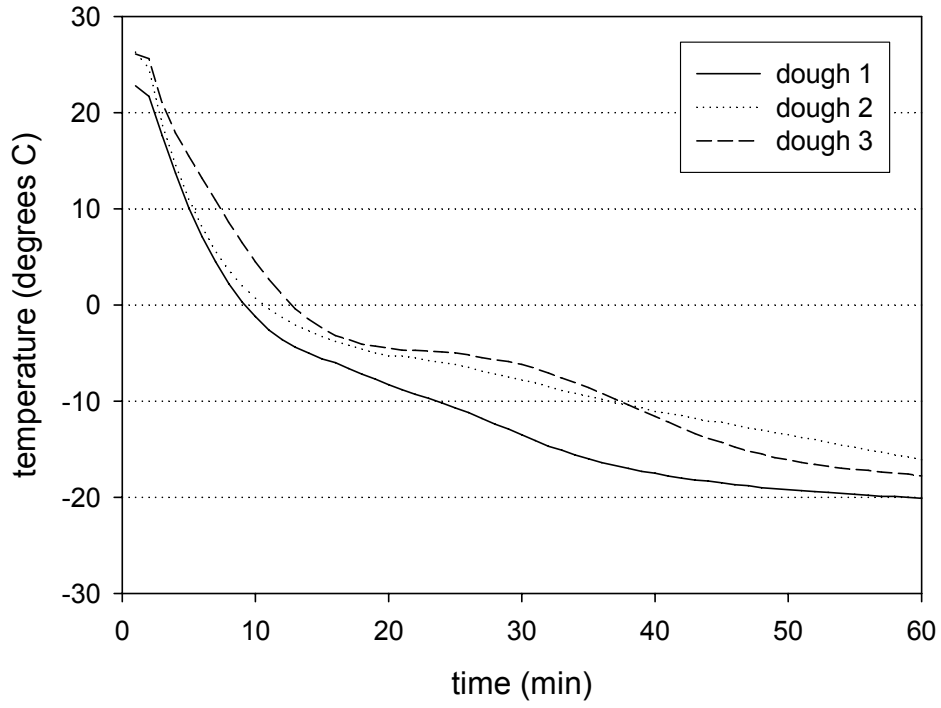
C. doughs were not frozen

D. thawed immediately after freezing

E. fermented in bulk (1kg batch) at room temperature (20˚C).

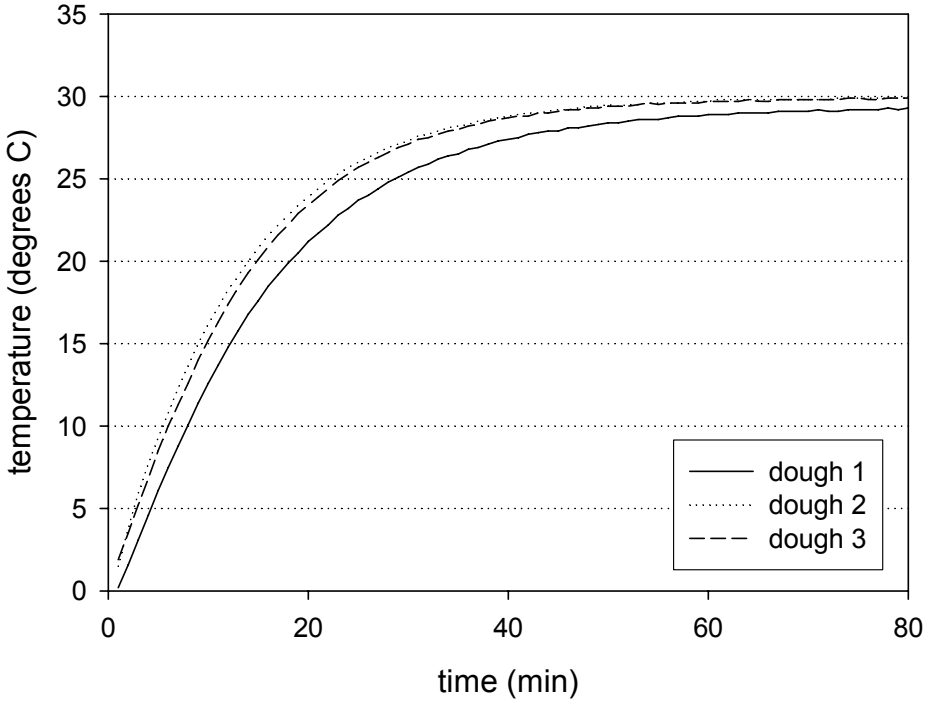
A4. Dough temperature profiles

Figure A4.1. Temperature profiles of 50g dough samples freezing in the refrigerated bath at -20˚C.



A batch of ten samples was frozen in the bath, with thermocouples measuring temperature in the sample closest to the cooling coil, the middle sample and the sample furthest from the cooling coil (doughs 1, 2 and 3 respectively).

Figure A4.2. Temperature profiles of 50 g dough samples transferred from an ice-water bath to the temperature-controlled room at 30˚C.



A5. Data from yeast metabolite extractions

Table A5.1. Yeast metabolites in dough made with 2% added glucose and 2% yeast, fermented at 30˚C for 5-90 minutes and extracted without heating.

fermentation time	extraction	assay	glucose	fructose	ethanol
min			mmol.(100g dough)$^{-1}$		
5	A	1	10.77		0.33
5	A	2	10.21		0.45
5	B	1	10.38	3.19	0.36
5	B	2	10.25	2.71	0.36
20	A	1	9.64		2.55
20	A	2	9.16		2.31
20	B	1	9.68		
20	B	2	9.26		
35	A	1	8.39		4.31
35	A	2	8.08		4.29
35	B	1	8.59	3.89	
35	B	2	8.97	2.95	
53	A	1	6.97		6.77
53	A	2	6.67		6.53
53	B	1	7.14		
53	B	2	7.08		
73	A	1	5.67	2.50	9.03
73	A	2	5.94	2.13	7.10
73	B	1	5.79		
73	B	2	5.57		
90	A	1	4.36		12.69
90	A	2	4.29		
90	B	1	4.62	2.50	13.88
90	B	2	4.37	2.24	14.04

Sugars in unyeasted doughs

Table A5.2. Sugars in unyeasted dough number 1 made with 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted without heating.

fermentation time	extraction	assay	glucose	fructose	sucrose	maltose
min			mmol.(100g dough)$^{-1}$			
0	A	1	1.46	3.06	6.37	2.51
0	A	2	0.88	2.89	6.34	1.75
0	B	1	1.29	2.57	6.38	
0	B	2	1.26	2.23	5.81	
30	A	1	1.45	2.53	6.61	3.34
30	A	2	1.45	2.49	6.08	3.41
30	B	1	1.27	2.08	6.53	
30	B	2	1.18	1.94	5.86	
60	A	1	0.78	1.25	6.70	3.05
60	A	2	0.81	1.09	6.39	3.00
60	B	1	0.84	1.40	7.23	
60	B	2	0.85	1.27	6.61	
90	A	1	1.28	1.88	6.94	3.81
90	A	2	1.21	1.75	6.52	3.84
90	B	1	1.19	1.76	6.98	
90	B	2	1.09	2.17	6.34	
120	A	1	0.64	2.31	7.13	3.97
120	A	2	0.63	2.35	6.43	3.70
120	B	1	0.68	2.39	7.61	
120	B	2	0.67	2.47	7.15	
150	A	1	0.65	1.10	7.43	
150	A	2	0.66	0.96	7.36	
150	B	1	0.68	1.07	7.86	
150	B	2	0.67	1.02	7.16	
180	A	1	0.76	0.72	7.17	2.97
180	A	2	0.75	0.95	6.72	3.29
180	B	1	0.81	0.86	5.91	
180	B	2	0.79	0.90	5.33	

Table A5.3. Sugars in unyeasted dough number 2 made with 2% added sucrose, fermented at 30˚C for 0-120 minutes and extracted without heating.

fermentation time	assay	glucose	fructose	sucrose	maltose
min		mmol.(100g dough)$^{-1}$			
0	1	0.18	0.31	7.29	1.58
0	2	0.17	0.15	6.71	1.16
30	1	0.25	0.36	9.18	1.85
30	2	0.22	0.31	5.95	1.79
60	1	0.38	0.44	7.41	2.71
60	2	0.37	0.29	6.90	2.75
90	1	0.41	0.35	7.32	2.13
90	2	0.37	0.26	6.72	2.05
120	1	0.52	0.42	7.32	2.71
120	2	0.47	0.37	6.39	2.52

Table A5.4. Sugars in unyeasted dough number 1 made with 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted with heating at 85˚C for 1 h.

fermentation time	assay	glucose	fructose	sucrose	maltose
min		mmol.(100g dough)$^{-1}$			
0	1	0.18	0.27	7.95	2.18
0	2	0.17	0.27	8.24	1.68
60	1	0.39	0.41	8.14	2.63
60	2	0.38	0.41	8.17	2.29
120	1	0.54	0.45	7.89	2.78
120	2	0.53	0.48	7.93	2.59
180	1	0.75	0.66	8.15	2.59
180	2	0.72	0.59	8.58	1.89

Table A5.5. Sugars in unyeasted dough number 3 made with 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted with heating at 85˚C for 1 h.

fermentation time	assay	glucose	fructose	sucrose	maltose
min		mmol.(100g dough)$^{-1}$			
0	1	0.00	0.28	8.57	2.96
0	2	0.16	0.29	7.56	2.41
60	1	0.34	0.33	7.54	3.69
60	2	0.35	0.33	7.75	3.11
120	1	0.42	0.86	8.07	4.71
120	2	0.46	0.50	7.31	3.82
180	1	0.70	0.63	7.62	4.53
180	2	0.70	0.60	8.16	3.84

Table A5.6. Sugars in unyeasted dough number 4 made with 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted with heating at 85˚C for 1 h.

fermentation time	assay	glucose	fructose	sucrose
min		mmol.(100g dough)$^{-1}$		
0	1	0.21	0.23	8.46
0	2	0.22	0.19	7.60
60	1	0.36	0.19	8.46
60	2	0.37	0.02	8.21
120	1	0.59	0.57	8.75
120	2	0.57	0.48	7.55
180	1	0.69	0.57	8.04
180	2	0.64	1.07	7.51

Sugars in yeasted doughs

Table A5.7. Yeast metabolites in yeasted dough number 1.

fermentation time	extraction	assay	glucose	fructose	sucrose	maltose	ethanol
min			mmol.(100g dough)$^{-1}$				
0	A	1	7.27	10.27	0.09	2.85	1.67
0	A	2	6.82	9.47	0.00	2.78	1.67
0	B	1	7.31	10.11	0.47	2.03	1.66
0	B	2	7.34	9.23	0.32	1.72	1.65
30	A	1	5.81	9.10	0.61	3.75	5.54
30	A	2	5.80	8.40	0.22	3.32	5.59
30	B	1	6.33	10.00	0.78	3.38	5.96
30	B	2	6.06	9.94	0.03	3.43	5.89
60	A	1	4.39	7.76	0.98	2.89	11.03
60	A	2	4.10	7.48	0.74	2.50	11.10
60	B	1	4.57	9.04	1.27	3.51	11.78
60	B	2	4.46	8.83	0.86	3.53	11.89
90	A	1	3.12	6.67	1.19	3.05	15.49
90	A	2	3.01	6.65	1.02	2.86	15.44
90	B	1	3.27	6.93	1.30	3.04	16.53
90	B	2	3.08	6.88	1.53	2.89	16.76
120	A	1	2.89	5.46	1.40	2.26	20.85
120	A	2	2.77	5.04	1.34	2.43	20.64
120	B	1	2.92	5.52	1.36	2.58	25.76
120	B	2	2.82	5.33	0.99	2.49	25.66
150	A	1	2.43	4.29	1.39	2.52	26.64
150	A	2	2.51	4.32	1.22	2.41	29.22
150	B	1	2.93	4.70	1.55	2.32	31.42
150	B	2	2.64	5.08	1.81	2.24	31.48
180	A	1	2.25	3.55	0.92	2.42	30.23
180	A	2	1.60	3.87	1.14	2.59	28.78
180	B	1	2.17	3.70	1.54	2.63	24.21
180	B	2	2.13	3.48	1.72	2.68	24.21

Dough was made with 2% yeast and 2% added sucrose, fermented at 30°C for 0-180 minutes and extracted without heating.

Table A5.8. Yeast metabolites in yeasted dough number 2.

fermentation time	extraction	assay	glucose	fructose	sucrose	maltose	ethanol
min			mmol.(100g dough)$^{-1}$				
0	A	1	6.27	9.89	1.27	2.89	0.93
0	A	2	6.12	9.54	0.80	2.52	0.99
0	B	1	6.92	11.05	1.43		0.94
0	B	2	6.56	9.92	0.96		0.95
30	A	1	4.98	9.27	1.05		3.96
30	A	2	4.91	8.90	0.59		3.88
30	B	1	5.75	10.02	0.98		4.58
30	B	2	5.44	11.53	0.61		4.56
60	A	1	3.82	9.34	1.01	3.08	9.76
60	A	2	3.75	9.25	0.72	2.70	9.72
60	B	1	4.05	9.62	1.16		10.03
60	B	2	3.84	9.19	1.49		9.84
90	A	1	2.44	9.29	1.07		18.04
90	A	2	2.44	9.27	0.80		18.06
90	B	1	2.38	8.76	1.14		18.25
90	B	2	2.30	8.08	0.93		18.45
120	A	1	1.34	7.60	1.18	2.92	21.09
120	A	2	1.39	7.22	1.07	2.51	20.83
120	B	1	1.48	7.03	1.17		22.14
120	B	2	1.36	6.90	0.97		22.24
150	A	1	1.16	5.72	1.24		27.38
150	A	2	1.11	5.73	1.02		27.19
150	B	1	1.05	5.53	1.38		26.82
150	B	2	1.11	5.70	1.66		26.71
180	A	1	0.92	5.28	1.61	1.62	29.81
180	A	2	0.93	5.22	1.87	1.29	30.29
180	B	1	0.79	4.63	1.40		31.98
180	B	2	0.70	4.57	1.18		31.06

Dough was made with 2% yeast and 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted without heating.

Table A5.9. Yeast metabolites in yeasted dough number 3

fermentation time	extraction	assay	glucose	fructose	sucrose	ethanol
min			mmol.(100g dough)$^{-1}$			
0	A	1	5.83	7.70	1.31	1.99
0	A	2	6.34	7.49	1.93	1.95
0	B	1	6.76	10.33	0.95	
0	B	2	6.32	9.63	0.11	
30	A	1	3.79	7.72	0.55	8.35
30	A	2	3.78	8.25	1.31	8.27
30	B	1	4.79	11.61	0.92	
30	B	2	4.00	9.34	0.47	
60	A	1	2.63	7.25	1.13	11.64
60	A	2	2.62	7.71	1.38	11.71
60	B	1	2.91	8.39	1.01	
60	B	2	2.73	7.81	0.30	
90	A	1	1.01	4.81	1.32	19.13
90	A	2	1.08	5.18	1.68	18.71
90	B	1	1.31	6.01	0.97	
90	B	2	1.30	5.70	0.89	
120	A	1	0.67	3.52	1.25	23.25
120	A	2	0.72	4.19	1.58	22.68
120	B	1	0.92	4.20	1.09	
120	B	2	0.90	3.97	0.78	
150	A	1	0.46	2.29	1.15	26.89
150	A	2	0.31	2.47	1.28	26.52
150	B	1	0.68	2.73	0.90	
150	B	2	0.65	2.84	0.86	
180	A	1	0.16	1.26	1.08	31.62
180	A	2	0.35	0.92	1.13	31.20
180	B	1	0.58	1.42	1.01	
180	B	2	0.56	1.39	1.33	

Dough was made with 2% yeast and 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted without heating.

Table A5.10. Sugars in yeasted dough number 1.

fermentation time	assay	glucose	fructose	sucrose	maltose
min		mmol.(100g dough)$^{-1}$			
0	1	6.50	12.53	1.73	3.97
0	2	6.23	12.25	1.09	3.21
30	1	4.62	11.20	0.50	
30	2	5.10	12.98	0.96	
60	1	3.31	10.70	0.67	3.54
60	2	3.13	10.09	0.30	3.42
90	1	1.98	8.58	0.52	
90	2	1.88	7.96	0.00	
120	1	0.87	5.57	0.36	4.25
120	2	0.85	5.85	0.25	3.81
150	1	0.59	4.66	0.18	
150	2	0.55	4.44	0.11	
180	1	0.59	2.95	0.00	3.85
180	2	0.43	2.93	0.00	3.39

Dough was made with 2% yeast and 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted with heating at 85˚C for 1 h.

Table A5.11. Sugars in yeasted dough number 2.

fermentation time	assay	glucose	fructose	sucrose	maltose
min		mmol.(100g dough)$^{-1}$			
0	1	7.35	10.47	0.51	3.00
0	2	7.12	9.88	0.26	2.52
30	1	5.77	10.94	0.25	
30	2	5.66	10.24	0.00	
60	1	4.25	9.01	0.46	3.68
60	2	3.54	9.52	0.27	3.08
90	1	2.15	8.37	0.10	
90	2	2.09	7.49	0.32	
120	1	0.99	6.48	0.35	3.98
120	2	0.93	6.20	0.22	3.23
150	1	0.49	3.81	0.27	
150	2	0.45	4.04	0.05	
180	1	0.33	2.88	0.07	3.10
180	2	0.32	2.88	0.19	2.86

Dough was made with 2% yeast and 2% added sucrose, fermented at 30˚C for 0-180 minutes and extracted with heating at 85˚C for 1 h.

Table A5.12. Sugars in yeasted dough number 3.

time	assay	glucose	fructose	sucrose
min		mmol.(100g dough)$^{-1}$		
0	1	5.84	11.66	1.26
0	2	5.71	10.25	0.72
30	1	3.73	8.99	0.86
30	2	3.55	9.80	0.70
60	1	2.53	8.79	0.70
60	2	2.34	8.79	0.64
90	1	0.88	5.89	0.31
90	2	0.86	5.86	0.26
120	1	0.48	3.97	0.14
120	2	0.46	3.94	0.16
150	1	0.33	2.51	0.16
150	2	0.33	2.37	0.15
180	1	0.24	1.10	0.15
180	2	0.22	1.05	0.18
210	1	0.17	0.43	0.16
210	2	0.15	0.39	0.11

Dough was made with 2% yeast and 2% added sucrose, fermented at 30˚C for 0-210 minutes and extracted with heating at 85˚C for 1 h.

Frozen, stored and thawed doughs

Fifty-gram samples of dough number 3, made with 2% sucrose and 2% yeast was split into four streams:

- unfrozen
- unfermented, frozen and stored 48 h at -20˚C then thawed at 0˚C and fermented at 30˚C.
- pre-fermented 30 minutes at 30˚C, frozen and stored 48 h at -20˚C then thawed at 0˚C and fermented at 30˚C
- pre-fermented 90 minutes at 30˚C, frozen and stored 48 h at -20˚C then thawed at 0˚C and fermented at 30˚C.

Doughs were extracted without heating at regular intervals during post-thaw fermentation and extracts assayed for yeast metabolites. Results from the 'unfrozen' stream are shown in Table A5.9.

Table A5.13. Yeast metabolites in unfermented frozen-thawed dough.

fermentation time	extraction	assay	glucose	fructose	sucrose	ethanol
min			mmol.(100g dough)$^{-1}$			
0	A	1	5.43	8.04	0.75	4.02
0	A	2	5.73	9.01	0.84	3.95
0	B	1	5.98	10.57	0.95	
0	B	2	5.95	9.83	0.17	
30	A	1	4.85	8.18	0.00	4.80
30	A	2	5.07	9.60	1.04	4.97
30	B	1	5.45	10.71	0.81	
30	B	2	5.30	11.63	0.24	
60	A	1	3.52	8.03	0.75	9.00
60	A	2	3.62	8.66	1.06	8.96
60	B	1	3.92	9.68	0.64	
60	B	2	3.81	9.19	0.31	
90	A	1	1.89	7.16	0.76	14.97
90	A	2	2.05	7.05	0.87	14.76
90	B	1	2.17	7.47	0.65	
90	B	2	2.11	7.25	0.47	
120	A	1	1.27	6.93	1.19	17.49
120	A	2	1.31	6.26	1.37	17.04
120	B	1	1.98	7.97	1.14	
120	B	2	1.70	6.40	0.88	

Dough was made with 2% sucrose and 2% yeast, frozen immediately after mixing, stored 48h at -18˚C, thawed and fermented at 30˚C.

Table A5.14. Yeast metabolites in frozen-thawed dough prefermented 30 minutes.

fermentation time	extraction	assay	glucose	fructose	sucrose	ethanol
min			mmol.(100g dough)$^{-1}$			
0	A	1	3.44	9.27	0.67	9.33
0	A	2	3.50	8.77	1.01	9.33
0	B	1	3.65	9.86	0.80	
0	B	2	3.44	8.98	0.31	
30	A	1	2.73	7.20	0.56	10.64
30	A	2	2.91	7.05	1.17	10.54
30	B	1	2.97	8.94	0.70	
30	B	2	2.93	8.82	0.50	
60	A	1	1.72	5.56	0.99	15.23
60	A	2	1.86	6.15	1.33	15.10
60	B	1	1.94	7.44	0.78	
60	B	2	1.88	7.76	0.00	
90	A	1	1.25	4.97	1.09	17.65
90	A	2	1.28	5.37	0.80	17.61
90	B	1	1.48	6.65	0.91	
90	B	2	1.46	6.79	0.99	
120	A	1	0.72	3.84	0.78	24.26
120	A	2	0.71	4.11	1.04	23.07
120	B	1	0.93	3.85	0.96	
120	B	2	0.91	4.32	0.86	

Dough was made with 2% sucrose and 2% yeast, prefermented 30 minutes at 30˚C, frozen, stored 48h at -18˚C, thawed and fermented at 30˚C.

Table A5.15. Yeast metabolites in frozen-thawed dough prefermented 90 minutes.

fermentation time	extraction	assay	glucose	fructose	sucrose	ethanol
min			mmol.(100g dough)$^{-1}$			
0	A	1	1.20	4.64	1.97	18.74
0	A	2	0.94	4.81	1.72	18.57
0	B	1	1.15	5.67	2.04	
0	B	2	1.13	5.30	1.92	
30	A	1	1.38	5.56	1.88	20.89
30	A	2	1.07	5.16	1.95	20.14
30	B	1	1.26	5.88	2.36	
30	B	2	1.25	5.51	2.12	
60	A	1	0.81	5.14	1.99	27.12
60	A	2	0.88	4.52	2.12	26.49
60	B	1	0.96	4.13	2.15	
60	B	2	0.92	3.95	1.86	
90	A	1	0.56	3.32	1.74	27.91
90	A	2	0.88	3.84	2.23	27.05
90	B	1	0.84	3.61	1.97	
90	B	2	0.83	3.56	1.77	
120	A	1	0.78	3.52	2.15	29.90
120	A	2	0.80	3.59	2.32	27.38
120	B	1	1.06	3.41	2.18	
120	B	2	1.03	3.18	1.84	

Dough was made with 2% sucrose and 2% yeast, prefermented 90 minutes at 30˚C, frozen, stored 48h at -18˚C, thawed and fermented at 30˚C.

Doughs with 2% glucose and added ethanol

Table A5.16. Glucose and ethanol in yeasted dough made with 2% added glucose and 5 ml.kg^{-1} added 95% ethanol.

fermentation time	extraction	assay	glucose	ethanol
min			mmol.(100g dough)$^{-1}$	
0	A	1	9.44	10.05
0	A	2	8.76	10.03
0	A	3	8.37	
0	A	4	8.88	
0	B	1		9.83
0	B	2		9.65
0	B	3	8.98	
0	B	4	8.50	
30	A	1	8.60	13.32
30	A	2	8.25	13.26
30	A	3	7.76	
30	A	4	7.41	
30	B	1		13.24
30	B	2		13.20
30	B	3	8.54	
30	B	4	7.79	
60	A	1	6.40	17.84
60	A	2	6.21	18.19
60	A	3	6.10	
60	A	4	6.20	
60	B	1		18.35
60	B	2		17.62
60	B	3	5.61	
60	B	4	5.28	
90	A	1	4.23	22.47
90	A	2	3.84	22.51
90	A	3	4.01	
90	A	4	3.96	
90	B	1		24.53
90	B	2		24.18
90	B	3	4.03	
90	B	4	3.88	

Table A5.17. Glucose and ethanol in yeasted dough made with 2% added glucose and 10 ml.kg^{-1} added 95% ethanol.

fermentation time	extraction	assay	glucose	ethanol
min			mmol.(100g dough)$^{-1}$	
0	A	1	9.36	17.68
0	A	2	9.33	18.71
0	A	3	9.18	
0	A	4	9.33	
0	B	1		18.01
0	B	2		17.44
0	B	3	10.27	
0	B	4	10.24	
30	A	1	8.81	23.05
30	A	2	8.87	21.89
30	A	3	8.54	
30	A	4	9.03	
30	B	1		21.10
30	B	2		21.08
30	B	3	9.07	
30	B	4	8.89	
60	A	1	6.24	30.81
60	A	2	6.26	31.05
60	A	3	5.98	
60	A	4	5.64	
60	B	1		25.26
60	B	2		24.85
60	B	3	6.14	
60	B	4	6.01	
90	A	1	4.55	32.64
90	A	2	4.44	32.80
90	A	3	4.55	
90	A	4	4.45	
90	B	1		30.58
90	B	2		30.19
90	B	3	3.94	
90	B	4	3.82	

Table A5.18. Glucose and ethanol in yeasted dough made with 2% added glucose and 20 ml.kg^{-1} added 95% ethanol.

time	assay	glucose	ethanol
min		mmol.(100g dough)$^{-1}$	
0	1	9.79	33.56
0	2	9.85	33.30
30	1	8.92	35.53
30	2	9.15	34.12
60	1	8.17	34.22
60	2	8.20	35.49
90	1	6.74	44.12
90	2	6.88	44.78

A6. Microsystem model program code

```
%A Matlab version 6.5 program to model diffusion of glucose and ethanol
%around a yeast cell metabolising reductively in dough aqueous phase
%November 2005
%Simon Miller

function []=dough_sim_1d()

clear all
close all

%independent variables
global t;   %time step number
global j;   %space step number

%dependent variables
global s;   %an array of sugar concentrations - a row for each time and a column for each space point
global e; %an array of ethanol concentrations
global Gr;          %rate of sugar consumption, mol/L/time step
global Er;          %rate of ethanol production, mol/L/time step

%system inputs
global gi;                  %initial glucose concentration, mol/L
global ei;                  %initial ethanol concentration, mol/L
global Nt;                  %number of time steps
global Nj;                  %number of space steps
global L;                   %size of the space domain, metres
global Ds;                  %diffusion coefficient for sugar, m2/s
global De;                  %diffusion coefficient of ethanol, m2/s
global dx;                  %size of space step, metres
global dt;                  %size of time step, minutes
global Gr_initial;          %initial rate of glucose consumption, mol/L.time step
global Er_initial;          %initial rate of ethanol production, mol/L.time step
global Yeg;                 %yield of ethanol from sugar, mol/mol

%computational variables
global e_array;             %array of coefficients for ethanol concentrations
global s_array;             %array of coefficients for sugar concentrations
global Rs;
global Re;
global yst_zone_hi  %number of the outermost point in the yeast cell zone

% system inputs
L = 0.0223e-3;
ei = 0;                     %assumption that no ethanol is initially present
gi = 0.432;
De = 2.68e-12;
Ds = 4.96e-12;
Nt = 600;
Nj = 50;
dt = 2;
dx = L/Nj;
Yeg = 1.82;
Gr_initial=0.00276;

yst_zone_hi=round(.003e-3/L*Nj);

if yst_zone_hi==1
        error('SPACE MESH TOO COARSE, INCREASE Nj');
end
```

```
Gr_initial = Gr_initial*[Nj/(yst_zone_hi)];
Gr_initial = Gr_initial/60*dt;
Er_initial = Yeg*Gr_initial;
Gr=Gr_initial;
Er=Er_initial;

Rs=dt*Ds/(dx^2);
Re=dt*De/(dx^2);

%matrix is initialised at uniform concentration, gi or ei.
s(1,1:Nj)=gi;
e(1,1:Nj)=ei;

define_coeff_arrays;

for j=1:Nt
        s_temp=s(j,:);
        e_temp=e(j,:);
        s_temp(1:yst_zone_hi)=s_temp(1:yst_zone_hi)-Gr;
        e_temp(1:yst_zone_hi)=e_temp(1:yst_zone_hi)+Er;

   RHS_sugar(1) = s_temp(1) + Rs*(s_temp(2)-s_temp(1));
   for i=2:Nj-1
      RHS_sugar(i)=s_temp(i) + Rs/2*(s_temp(i+1)-2*s_temp(i)+s_temp(i-1));
   end
   RHS_sugar(Nj) = s_temp(Nj) + Rs/2*(2*s_temp(Nj-1)-2*s_temp(Nj));
   s(j+1,:)= tri_diag(s_array,RHS_sugar);

        RHS_ethanol(1) = e_temp(1) + Re/2*(2*e_temp(2)-2*e_temp(1));
        for i=2:Nj-1
                RHS_ethanol(i)=e_temp(i) + Re/2*(e_temp(i+1)-2*e_temp(i)+e_temp(i-1));
        end
        RHS_ethanol(Nj) = e_temp(Nj) + Re/2*(2*e_temp(Nj-1)-2*e_temp(Nj));
        e(j+1,:)= tri_diag(e_array,RHS_ethanol);

        for i=1:Nj
                if s(j+1,i)<0
                        s(j+1,i)=0;
                        e(j+1,i)=e(j,i);
                end
        end
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%plots graphs of sugar and ethanol concentration, space and time axes

max_rows=20;
max_cols=20;
time_jump_size=floor((Nt+1)/max_rows);
space_jump_size=floor(Nj/max_cols);

s_red_time(1,:)=s(1,:);

for j=1:max_rows-1
        s_red_time(j+1,:)=s(time_jump_size*j,:);
end

s_red(:,1)=s_red_time(:,1);

for j=1:max_cols-1
        s_red(:,j+1)=s_red_time(:,space_jump_size*j);
```

```
end

e_red_time(1,:)=e(1,:);

for j=1:max_rows-1
        e_red_time(j+1,:)=e(time_jump_size*j,:);
end

e_red(:,1)=e_red_time(:,1);

for j=1:max_cols-1
        e_red(:,j+1)=e_red_time(:,space_jump_size*j);
end

xgrid=(0:1000000*L/(max_cols-1):1000000*L).';
ygrid=(0:Nt*dt/60/max_rows:(Nt-1)*dt/60);
cmap=[zeros(64,1) zeros(64,1) zeros(64,1)];

mesh(xgrid,ygrid,s_red);
shading interp
view(-145, 18)
xlabel('distance (micrometres)', 'fontsize', 14);
ylabel('time (min)', 'fontsize', 14);
zlabel('sugar (mol/L)', 'fontsize', 14);
set(gcf,'Color','w');
set(gcf,'Units','centimeters');
set(gcf,'position',[5 5 13 10]);
set(gcf,'colormap',cmap);
set(gca,'position',[.15, .15, .75, .75]);
set(gca,'color',[.85 .85 .85]);

figure
mesh(xgrid,ygrid,e_red);
shading interp
view(-145, 18);
xlabel('distance (micrometres)', 'fontsize', 14);
ylabel('time (min)', 'fontsize', 14);
zlabel('ethanol (mol/L)', 'fontsize', 14);
set(gcf,'Color','w');
set(gcf,'Units','centimeters');
set(gcf,'position',[5 5 13 10]);
set(gcf,'colormap',cmap);
set(gca,'position',[.15, .15, .75, .75]);
set(gca,'color',[.85 .85 .85]);


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% adapated from 'neumann_heat_cn.m' obtained from Mary Pugh at University of Toronto
% http://www.math.toronto.edu/mpugh/Teaching/AppliedMath/Spring99/Heat/neumann_heat_cn.m

function []=define_coeff_arrays()

global s_array e_array Nj Rs Re

s_array=zeros(Nj,Nj);
s_array(1,1) = 1+Rs;
s_array(1,2) = -Rs;
for i=2:Nj-1
        s_array(i,i-1) = -Rs/2;
        s_array(i,i) = 1+Rs;
        s_array(i,i+1) = -Rs/2;
end
```

```
s_array(Nj,Nj) = 1+Rs;
s_array(Nj,Nj-1) = -Rs;

e_array=zeros(Nj,Nj);
e_array(1,1) = 1+Re;
e_array(1,2) = -Re;
for i=2:Nj-1
        e_array(i,i-1) = -Re/2;
        e_array(i,i) = 1+Re;
        e_array(i,i+1) = -Re/2;
end
e_array(Nj,Nj) = 1+Re;
e_array(Nj,Nj-1) = -Re;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% adapated from 'tri_diag.m' obtained from Mary Pugh at University of Toronto
% http://www.math.toronto.edu/mpugh/Teaching/AppliedMath/Spring99/Heat/tri_diag.m

function x = tri_diag(A,rhs)

[n,n] = size(A);

for i=1:n
        b(i)=A(i,i);
end
for i=1:n-1
        c(i)=A(i,i+1);
end
for i=2:n
        a(i) = A(i,i-1);
end

% solve for the entries of L and U sot that LU =A
beta(1) = b(1);
for j=2:n
        alpha(j) = a(j)/beta(j-1);
        beta(j) = b(j)-alpha(j)*c(j-1);
end

% solve Ly=b
y(1)=rhs(1);
for j=2:n
        y(j)=rhs(j)-alpha(j)*y(j-1);
end

% solve Ux = y
x(n) = y(n)/beta(n);
for j=1:n-1
        x(n-j) = (y(n-j)-c(n-j)*x(n-j+1))/beta(n-j);
end
```

A7. Macrosystem model program code

Matlab program ‘macrosystem_model.m’

```
% A Matlab release 13 program to model glucose and fructose uptake and
% ethanol production in fermenting dough.
% code requires 'yeast.m'
% Simon Miller
% November 2005

clear all
close all

global Gi Fi Mi Ei                  % initial concentrations of glucose, fructose, maltose, ethanol, mol/L
global G F M E                      % concentrations of glucose, fructose, maltose, ethanol, mol/L
global X                            % biomass concentration, g/L
global Tfin                         % length of simulated fermentation, hours
global Yeg Yef Yem                  % molar yields of ethanol from glucose, fructose and maltose, mol/mol
global Kg Kf Ki VmaxG VmaxF         % kinetic parameters

%kinetic parameters adjusted for best fit
Kg=0.05;        %Michaelis-Menten constant for glucose uptake, mol/L
Kf=0.001;       %Michaelis-Menten constant for fructose uptake, mol/L
Ki=0.0005;    %competitive inhibition constant for inhibition of fructose uptake by glucose, mol/L

%experimentally-determined parameters
VmaxG=0.00653; %maximal glucose uptake rate, mol/L.h
VmaxF=0.00626; %maximal fructose uptake rate, mol/L.h
Yeg=1.82;
Yef=1.86;
X=25.4;
Gi=0.250;
Fi=0.389;
Ei=0.0559;
Mi=0;

Tfin=3;

%initial conditions
yi=[Gi,Fi,Mi,Ei];

%numerical solution using matlab ODE solver 'ode45'
tspan=[0 Tfin];
options=odeset('refine', 1);
[t,y]=ode45('yeast', tspan, yi,options);

%convert from mol/L to mmol/(100g dough) dough
y=y*0.02576*1000;

%output sugar and ethanol concentrations
store=[t*60,y(:,1:4)];
save 'Z:\output.out' store -ASCII;

figure
plot(t,y(:,1:2));
legend('predicted glucose', 'predicted fructose',1);
figure
plot(t,y(:,4));
legend('predicted ethanol',2);
```

Matlab program ‘yeast.m’

```
% A Matlab version 6.5 program to model glucose and fructose uptake and
% ethanol production in fermenting dough
% code is accessed by 'macrosystem_model.m'
% Simon Miller
% November 2005

function odes = yeast(t,y)

global Gi Fi Mi Ei          % initial concentrations of glucose, fructose, maltose, ethanol, mol/L
global G F M E              % concentrations of glucose, fructose, maltose, ethanol, mol/L
global X                    % biomass concentration, g/L
global Tfin                 % length of simulated fermentation, hours
global Yeg Yef Yem          % molar yields of ethanol from glucose, fructose and maltose
global Kg Kf Ki VmaxG VmaxF % kinetic parameters

G=y(1);
F=y(2);
M=y(3);
E=y(4);

odes=zeros(4,1);

if G<= 1e-5
        G=0;
        odes(1)=0;
else
odes(1)=-1*X*[VmaxG*G/(G+Kg)];          %dG/dt, mol/L.h
end

if F<= 1e-5
        F=0;
        odes(2)=0;
else
odes(2)=-1*X*VmaxF*F/(F+Kf*(1+G/Ki));   %dF/dt, mol/L.h
end

odes(3)=0;                              %dM/dt, mol/L.h
odes(4)=-1*odes(1)*Yeg - odes(2)*Yef;   %dE/dt, mol/L.h
```