ELSEVIER

International Journal of Food Microbiology 35 (1997) 109–116

International Journal of Food Microbiology

Optimisation of methodology for enumeration of xerophilic yeasts from foods

S. Andrews*, H. de Graaf, H. Stamation

School of Chemical Technology, University of South Australia, The Levels, S.A. 5095, Australia

Abstract

Xerophilic yeasts grow in intermediate moisture foods (a_w, 0.65–0.85) such as sugar syrups, fruit concentrates, jams and brines. Non-osmophilic yeasts are enumerated by diluting in 0.1% peptone and then plated onto media such as malt extract or glucose yeast extract agar. In the presence of moulds the yeasts are enumerated in dichloran rose bengal chloramphenicol agar (DRBC). These procedures were demonstrated to be unsatisfactory for the enumeration of xerophilic yeasts in low a_w foods. Investigations using pure cultures of xerophilic yeasts as well as naturally contaminated apple juice concentrates and glacé cherries have shown that a reduced a_w diluent, in particular 30% w/w glycerol in combination with tryptone 10% glucose yeast extract agar (TGY) optimises the recovery of the yeasts, especially sublethally injured cells. The inclusion of sodium chloride in either the diluents or the culture media was not necessary to optimise the recovery of *D. hansenii* growing in 20% sodium chloride broths. © 1997 Elsevier Science B.V.

Keywords: Xerophilic yeasts; Food; Diluent; Culture media; Glycerol

1. Introduction

Xerophilic yeasts grow below 0.85 a_w and include *Zygosaccharomyces rouxii*, *Z. bailii*, *Z. bisporus*, *Schizosaccharomyces pombe*, *Debaryomyces hansenii* and *Saccharomyces cerevisiae* (Pitt, 1975; Tilbury, 1980; Beuchat, 1987). Most of these yeasts are known for fermentative spoilage of products containing high sugar concentrations (Tilbury, 1980; Pitt and Hocking, 1985). In addition, *D. hansenii* has the ability to grow in salt concentrations as high as 24%, but more commonly 15–20% sodium chloride (Pitt and Hocking, 1985). Some of these species are also preservative resistant with *Z. bailii* and *Schiz. pombe* being the two most commonly isolated from spoiled foods (Pitt and Hocking, 1985). The most xerophilic of the yeasts is *Z. rouxii* which has been shown to grow at water activities as low as 0.65 (Pitt and Hocking, 1985; Beuchat, 1987).

* Corresponding author. Tel.: +61 8 3023147; fax: +61 8 3023668.

PII S0168-1605(96)01209-3

Table 1
Recovery of sublethally injured xerophilic yeasts on various enumeration media using 30% glycerol as a diluent

Yeast[a]	Log_{10} (viable count)				$CI_{95\%}$
	DRBC	MY3OG	DG18	TGY	
Z.rouxii FFR3669	3.83*	4.40	4.40	4.40	3.98–4.54
Z.bailii FFR2227	4.85*	5.81	5.89	5.67	5.09–6.03
D. hansenii FFR2577	5.43*	6.96	6.91	6.98	5.82–7.32
S. cerevisiae USA931	5.02*	5.66	5.77	5.66	5.19–5.86
Schiz. pombe USA932	3.95*	5.41	5.54	5.82	4.36–6.00

[a] Yeast cultures heated at 48°C for 5 min, stored at −20°C for 7 days, then heated at 48°C for 5 min.
* Below 95% confidence limits.

Xerophilic yeasts accumulate high concentrations of glycerol as a response to low water activities (Brown, 1976, 1978). The glycerol is known to protect enzymes at low a_w and to serve as an osmoregulator under water stress. If rapid changes in the extracellular osmotic pressure occur, such as in a diluent, this may cause cell wall rupture and death (Gould, 1989). Stressed or sublethally injured cells are more likely to have a reduced osmoregulatory capacity and be less able to survive changes in osmotic pressure (Gould, 1989). Hence, in analysing intermediate moisture foods, which have an a_w in the range 0.60–0.85 (Leistner and Rödel, 1976), yeasts present may be inactivated by osmotic shock when these products are diluted in high a_w diluents such as 0.1% peptone. Sublethally injured yeasts are less likely to survive these procedures. This paper describes some studies using a range of glycerol concentrations as an osmoprotectant when preparing dilutions of intermediate moisture foods for enumeration of xerophilic yeasts. In particular, the role of glycerol is investigated for sublethally injured yeasts.

For enumerating yeasts from low a_w foods, it is not always essential to use a selective medium. If a food has a water activity of 0.85 or less and shows signs of spoilage, then the dominant yeasts recovered must be xerophilic. However, if an intermediate moisture food is being screened for the presence of xerophilic yeasts, a selective medium is desirable. The disadvantage of a selective medium is that an incubation period of up to 28 days may be required (Tilbury, 1980).

This study sought to determine whether media higher than 0.95 a_w could be used for rapid detection of xerophilic yeasts. The media used included dichloran 18% glycerol agar (DG18) used extensively for the enumeration of xerophilic fungi from foods (Hocking et al., 1992), which is approximately 0.955 a_w; malt extract 30% glucose (MY3OG), developed as a glucose based medium of similar water activity to DG18; and tryptone 10% glucose yeast extract agar (TGY) which evolved out of reports that xerophilic yeasts grow more vigorously in 10% w/v rather than 2% w/v sugar concentrations (Scarr and Rose, 1965). These media were evaluated against dichloran rose bengal chloramphenicol agar (DRBC) (King et al., 1979) the recommended medium for enumeration of normal yeasts in the presence of both bacteria and moulds (Pitt et al., 1992). This paper summarises studies carried out with a number of pure cultures of typical xerophilic yeasts, including *Z. rouxii*, *Z. bailii*, *D. hansenii*, *Schiz. pombe*, and *S. cerevisiae* on these reduced a_w media. In addition, naturally contaminated reduced a_w foods were included. The effectiveness of the different concentrations of glycerol in the diluents were compared to 0.1% peptone, the diluent recommended for enumeration of yeasts from high a_w foods (Hocking et al., 1992). Based on the studies reported here, a procedure for optimum recovery of xerophilic yeasts from intermediate moisture foods is proposed.

Table 2
Recovery of xerophilic yeasts from apple juice concentrates and glace cherries on various enumeration media using 30% glycerol as a diluent

Sample	Log_{10} (viable count)				CI_{95}
	DRBC	MY30G	DG18	TGY	
AJC-9	5.41*	5.95	5.90	5.88	5.54 6.03
AJC-10	4.71*	5.79	5.73	5.50	4.95 5.92
AJC-12	5.00*	5.69	5.63	5.57	5.16 5.79
AJC-14	5.08*	5.77	5.97	5.82	5.27 6.05
AJC-15	5.42*	6.09	6.09	5.97	5.58 6.18
AJC-18	5.40*	5.93	5.84	5.76	5.51 5.96
Glacé cherries	Nil*	4.94	3.79	4.11	3.61 4.95

AJC, apple juice concentrates.
* Below 95% confidence limits.

2. Materials

2.1. Yeasts

The following species were studied: *Debaryomyces hansenii* FRR 1570 (FRR denotes the culture collection of CSIRO Division of Food Science, North Ryde, NSW, Australia) from salted fish eggs; *D. hansenii* FRR 2577 from salted fish; *Saccharomyces cerevisiae* USA 931 (USA denotes the culture collection of University of South Australia, Adelaide, SA, Australia) from grape juice; *Schizosaccharomyces pombe* USA 932 from fruit based cordial; *Zygosaccharomyces bailii* FRR 1299 from spoiled passion-fruit soft drink; *Z. bailii* FRR 2227 from raspberry cordial; *Zygosaccharomyces rouxii* FRR 3669 from table wine; and *Z. rouxii* FRR 3681 from high fructose corn syrup.

2.2. Media

The media used were DRBC ($a_w > 0.997$) (Pitt and Hocking, 1985); DG18 (a_w, 0.955) (Pitt and Hocking, 1985); malt extract yeast extract 30% glucose agar (MY30G) ($a_w \sim 0.96$), and TGY ($a_w \sim 0.987$). The composition of MY30G comprises: malt extract, 1 g; yeast extract, 3.5 g; agar, 14 g; distilled water to 700 g; glucose, 300 g. The composition of TGY is: tryptone, 5 g; yeast extract, 5 g; glucose, 100 g; agar, 15 g; distilled water to 1 l (Samson et al., 1992). The MY30G was sterilized by steaming for 30 min on two successive days, DRBC and DG18 were autoclaved at 121°C for 15 min and TGY autoclaved at 110°C for 10 min.

Yeasts were also grown in MY60G broths (malt extract, 8 g; yeast extract, 2 g; glucose, 600 g; distilled water, 400 g) for recovery experiments. Some cultures of *D. hansenii* were also grown in malt extract broth (Oxoid, CM57) supplemented with 15 or 20% sodium chloride.

2.3. Diluents

A number of diluents were assessed in this study including 0.1% bacteriological peptone (Oxoid, L37); 10% w w glucose A.R.; 5, 10, 20 and 30% w w glycerol A.R.; 0.9, 3.0 and 20% w/w sodium chloride A.R. All diluents were sterilized for 15 min at 121°C.

2.4. Samples

Six samples of apple juice concentrate (70° Brix) which were showing signs of fermentative spoilage, were diluted and enumerated on the four test media. A sample of glacé cherries (68° Brix) in a sealed plastic pouch which was grossly distorted due to gas production, was also diluted and cultured.

Table 3
Effect of various diluents on the recovery of xerophilic yeast suspensions after being heated at 48–53°C for 5 min

Yeast	Log_{10} (viable count)			$CI_{95\%}$
	Peptone (0.1%)	Glucose (10%)	Glycerol (30%)	
Z rouxii FFR3681	2.70	3.77	3.66	2.71–4.05
Z.rouxii FFR3669	4.23	4.42	5.37	3.98–5.36
Z. bailii FFR2227	4.89	4.92	5.77	4.62–5.76
Z.bailii FFR1229	4.86	4.99	5.65	4.69–5.65
Schiz. pombe USA932	4.53	4.60	4.57	4.53–4.61

2.5. Growth in pure cultures

Z rouxii, *Z bailii*, *Schiz. pombe*, *S. cerevisiae* and *D. hansenii* were grown in 50% glucose yeast extract broths before being plated out onto the four test media DRBC, DG18, MY30G and TGY.

2.6. Stressed cultures

The yeast cultures were subcultured from the 50% glucose broths into 60% glucose broths and incubated. Stationary phase cultures were then subjected to 48°C for 5 min, cooled, stored at −20°C for 7 days, thawed and reheated at 48°C for 5 min to produce some sublethally injured xerophilic yeasts. These stressed cells were diluted in 30% glycerol, and plated onto the four test media.

2.7. Evaluation of diluents

Diluents of decreasing water activity were prepared using 5, 10, 20 and 30% w/w glycerol. These diluents were compared to 0.1% peptone, the reference diluent and 10% w/w glucose. To evaluate the role of diluents only one culture medium was required and TGY was selected because it produced the most rapid and typical growth for the yeasts.

2.8. Salt tolerant species

The two cultures of *D. hansenii* were grown in malt broths containing 15% w w NaCl and then transferred to 20% w w NaCl broths. These broths, plus heat stressed broths, were then diluted in 0.9, 3.0 and 20% w/w sodium chloride solutions. The broths were also diluted in 0.1% peptone, 5 and 30% glycerol which had similar water activities to the saline diluents.

3. Results

3.1. Growth of pure cultures

Preliminary investigations indicated that all species tested produced medium size colonies on TGY within 5 days at 25°C. In general, the growth of the yeasts on DG18 was more variable and weaker, colonies on MY30G were slower to develop and *Z. rouxii* grew very poorly on DRBC. When the 50% glucose broth cultures of the yeast were diluted in 0.1% peptone and plated out onto the four media, it was not possible to demonstrate any reproducible differences between the media for the enumeration of the yeasts, although DRBC recovered the lowest number for most of the yeast cultures.

3.2. Stressed cultures

The recovery rate of stressed cells was lower on DRBC (Table 1). The viable counts recorded on MY30G, DG18 and TGY were similar (Table 1), although the colonies were larger and more readily counted on TGY.

Table 4
Effect of various diluents on the recovery of xerophilic yeasts from apple juice concentrates and glace cherries

Sample	Log_{10} (viable count)			$CI_{95\%}$
	Peptone (0.1%)	Glucose (10%)	Glycerol (30%)	
AJC-9	5.27	5.54	5.88	5.21–5.91
AJC-10	4.76*	5.49	5.50	4.77–5.73
AJC-12	4.96	5.14	5.57	4.87–5.57
AJC-14	5.07*	5.75	5.82	5.09–6.01
AJC-15	5.49	5.64	5.97	5.41 5.99
AJC-18	5.26	5.62	5.76	5.26–5.84
Glacé cherries	2.64*	3.74	4.11	2.80–4.20

AJC, apple juice concentrates.
* Below 95% confidence limits.

3.3. Concentrated foods

Viable counts of apple juice concentrate were significantly lower on DRBC (Table 2) than on the other three media. The viable counts observed on the other three media were similar indicating that the spoilage yeast *Z. rouxii* could be recovered from a low a_w food equally well on MY30G, DG18 or TGY. A yeast from spoiled glacé cherries, identified later as *Z. bailii*, failed to grow on DRBC (Table 2) but grew very well on the other media, optimally on MY30G (Table 2).

These results indicate that recovery of xerophilic yeasts either from low a_w environments or sublethally injured cells can be successful on TGY, MY30G or DG 18 but not on DRBC.

3.4. Diluents

Preliminary experiments using yeast cultures grown in 60% glucose diluted in all the various test diluents showed no consistent trends for protection against osmotic shock. Similarly, stressed cultures of *Z. rouxii* which were frozen at −20°C for 7 days in 60% glucose broths, thawed and diluted in the various diluents showed no consistent differences in recovery. When the xerophilic yeasts were grown in 60% glucose broths and heated at 48°C for 5 min, diluted and plated onto TGY, it was possible to demonstrate that the lowest recovery of yeasts occurred with 0.1% peptone as the diluent (Table 3). This effect appeared to be more dramatic with *Z. rouxii* (Table 3). Similarly, when samples of apple juice concentrates or glacé cherries were homogenized and diluted in 0.1% peptone, 10% glucose or 30% glycerol, the poorest recovery of yeasts occurred in 0.1% peptone as the diluent (Table 4). Comparisons between 10% glucose and 30% glycerol do not show consistent differences, although the average counts with 30% glycerol diluent are 70–80% higher for both sets of results.

It was observed that for four of the test samples, increasing the glycerol concentration had a significant and consistent protective effect on the enumeration of yeasts from these osmotic environments (Table 5). The results for the heat stressed *Z. rouxii* FRR 3681, were very erratic, but this may have been due to the very low recovery rate of the yeasts after mild heat treatment.

3.5. Recovery of a salt tolerant yeast

In general, the counts for *D. hansenii* on TGY after dilution with 30% glycerol were higher than those observed with 0.9, 3.0 or 20% sodium chloride (Table 6). Furthermore, increasing the salt concentration in the diluent did not enhance the enumeration of either culture of *D. hansenii* despite previous growth in 20% sodium chloride (Table 6). For completeness, the addition of up to 10% sodium chloride in TGY was tested, but no beneficial effects were observed in terms of enu-

Table 5
Effect of various glycerol concentrations in diluents used to enumerate xerophilic yeasts

Diluent	Log_{10} (viable count)				
	Z. rouxii FFR 3669[a]	*Z. bailii* FFR 2227[a]	*Z. rouxii* FFR 3681[b]	Glacé cherries	Apple juice concentrate
Peptone (0.1%)	4.42*	5.16*	1.70*	2.64*	4.61
Glycerol (5%)	4.73	5.19	2.32	2.96	4.60
Glycerol (10%)	5.16	5.40	2.23	3.26	4.85
Glycerol (20%)	5.37	5.55	2.82**	3.87	5.10
Glycerol (30%)	5.57**	5.72**	2.36	4.11**	5.45**
$CI_{95\%}$	4.64–5.46	5.19–5.61	1.94–2.64	2.97–3.89	4.60–5.24

[a] Yeasts heated at 53°C for 5 min, stored −20°C for 7 days, heated 48°C for 5 min.
[b] Yeasts heated at 48°C for 5 min.
* Below 95% confidence limits.
** Above the limits.

meration or quality of colony characteristics for the salt enriched TGY compared to normal TGY.

4. Discussion

The xerophilic yeasts *Z. rouxii*, *Z. bailii*, *Schiz. pombe*, *D. hansenii* and *S. cerevisiae* all grew well on TGY to produce distinct colonies within 5 days at 25°C. TGY is simple to prepare and to sterilize in the autoclave at reduced pressure and may be used for the growth of all yeasts, not just the xerophiles. Yeasts may be enumerated from high a_w foods on TGY provided oxytetracycline or chloramphenicol is added to the medium to suppress the growth of bacteria (Hocking et al., 1992). For high a_w foods where yeasts must be enumerated in the presence of moulds, DRBC is preferred (Hocking et al., 1992).

Although the xerophilic yeasts grew on DRBC, growth was not as strong as that observed on TGY, in particular *Z. rouxii* grew weakly. Stress reduced the quantitative recovery of the yeasts on DRBC. The poor recovery was probably due to the low glucose concentration (1%) in the medium as well as the presence of rose bengal and dichloran. DRBC is not normally recommended for the enumeration of fungi from low a_w foods because some xerophilic fungi fail to grow (Hocking and Pitt, 1980). This study confirms that DRBC cannot be recommended for the examination of yeasts or fungi from low a_w commodities.

DG18 is the best medium currently available for enumeration of common xerophilic fungi (Hocking et al., 1992) and in this study was shown to support the growth of xerophilic yeasts as well. The quantitative recovery of the stressed osmophilic yeasts on DG18 was not significantly different from TGY, however the rate of colony formation on DG18 was slower because the medium contains only 1% glucose. The presence of 18% glycerol reduces the water activity to approximately 0.955 and helps to protect the xerophilic yeasts, but does not provide a substrate which some yeasts readily assimilate (Barnett et al., 1983).

MY30G supported the growth of the yeasts and recovered the stressed yeasts equally as well as TGY and DG18. The 30% glucose provided abundant substrate for the yeasts to grow but the reduced water activity slowed their growth rates. Compared to TGY, MY30G is more expensive to prepare and sterilisation of this medium by autoclaving may induce formation of furfural and other Maillard reaction products (Tilbury, 1980; Nagao et al., 1983; Shibamoto, 1983). These compounds are thought to be inhibitory to the growth of yeasts although Golden and Beuchat, 1992 were unable to demonstrate any inhibition on heat stressed *Z. rouxii*.

TGY was shown to be the best medium for rapid, distinct growth of xerophilic yeasts from intermediate moisture foods, including the recov-

Table 6
Effect of salt in diluents on the recovery of *D. hansenii* FRR1570 and FRR2577 grown in 20% sodium chloride malt broths

Diluent	Log_{10} (viable count)			
	D. hansenii FRR1570		*D. hansenii* FRR2577	
	Normal	Stressed	Normal	Stressed
Peptone (0.1%)	5.70	5.18*	6.78**	6.44
NaCl (0.9%)	5.69	5.21	6.54	6.31*
Glycerol (5%)	6.13**	5.36	6.10*	6.40
NaCl (3%)	5.46*	5.25	6.53	6.53**
Glycerol (30%)	6.30**	5.71**	6.73**	6.56**
NaCl (20%)	5.79	5.15*	6.59	6.54**
$CI_{95\%}$	5.67–6.09	5.19–5.52	6.36–6.70	6.38–6.52

* Below 95% confidence limits.
** Above the limits.

ery of sublethally injured yeasts. It is suggested that this medium be tested more extensively with other xerophilic yeasts to ascertain whether it can be universally recommended.

Based on the loss of yeast viability due to osmotic shock observed in this study, the enumeration of xerophilic yeasts from intermediate moisture foods in 0.1% peptone is not recommended. This study has demonstrated the protective effect of glycerol, especially 30% w w glycerol in the preparation of dilutions of yeasts from extremely low water activity (a_w, 0.81–0.82), or where the xerophilic yeasts were sublethally injured. In either situation, it is presumed that the presence of extracellular glycerol in the diluents helps to relieve the strain on the osmoregulatory capacity (Gould, 1989) of the yeast cells as they adjust from a low to high water activity.

Although 20% glucose or sucrose have been recommended as solutes in the diluents for enumeration of xerophilic yeasts (Tilbury, 1980), only 10% glucose was used in this study. In general 10% glucose (a_w, 0.99) was not as effective as 30% glycerol (a_w, 0.93) in protecting the yeasts, and this could be attributed either to the lower a_w for the glycerol solution or the difference in the solute molecule. Increased concentrations of glucose were not tested because of the inconvenience of sterilizing glucose solutions of greater than 20% w/w, and secondly, the possible presence of inhibitory products from the heating of the sugar solutions. Furthermore, a 45% w/w glucose solution, equivalent in a_w to 30% w/w glycerol, is a lot more viscous to handle, which does not enhance the procedure for making accurate uniformly dispersed dilutions.

A brief examination of cultures of *D. hansenii* demonstrated that although this yeast grows and proliferates in brine solutions, sodium chloride in diluents or culture media was not necessary for optimal recovery of this yeast, even when sublethally injured. Glycerol in the diluents was more protective than sodium chloride at equivalent water activities. *D. hansenii* is a halotolerant yeast but as observed in this study, it is definitely not an obligate halophile.

Based on the studies reported in this paper, it is recommended that xerophilic yeasts from intermediate moisture foods be enumerated by homogenisation and dilution in 30% w/w glycerol and then plated out onto TGY. The yeasts can be counted after 3–5 days incubation at 25°C. The yeasts developing on TGY may include normal yeasts if the low a_w food is not spoiled and is being screened for the yeast flora. In this case, the different yeasts need to be subcultured and identified. Additional studies are required to determine if higher levels of glycerol than 30% would be of value required to optimise the recovery of stressed xerophilic yeasts, however it is anticipated that effects will be minor.

References

Barnett, J.A., Payne, R.W. and Yarrow, D. (1983) Yeasts: characteristics and identification. Cambridge University Press, Cambridge.

Beuchat, L. (1987) Food and beverage mycology, 2nd edn. Avi Publications, New York.

Brown, A.D. (1976) Microbial water stress. Bacteriol. Rev. 40, 803–846.

Brown, A.D. (1978) Compatible solutes and extreme water stress in eukaryotic microorganisms. Adv. Microbial Physiol. 17, 181–242.

Golden, D.A. and Beuchat, L.R. (1992) Influence of method of sterilization and solute in the recovery medium on enumeration of heat-stressed *Zygosaccharomyces rouxii*. In: R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King (editors), Modern Methods in Food Mycology. Elsevier, Amsterdam, pp. 93–96.

Gould, G.W. (1989) Drying, raised osmotic pressure and low water activity. In: G.W. Gould (editor), Mechanisms of Action of Food Preservation Procedures. Elsevier, London, pp. 97–117.

Hocking, A.D. and Pitt, J.I. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Environ. Microbiol. 39, 488–492.

Hocking, A.D., Pitt, J.I., Samson, R.A. and King, A.D. (1992) Recommendations from the closing session of SMMEF II. In: R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King (editors), Modern Methods in Food Mycology. Elsevier, Amsterdam, pp. 359–364.

King, A.D., Hocking, A.D. and Pitt, J.I. (1979) Dichloran-rose bengal medium for enumeration and isolation of moulds from foods. Appl. Environ. Microbiol. 37, 959–964.

Leistner, L. and Rödel, W. (1976) The stability of intermediate moisture foods with respect to microorganisms. In: R. Davies, G.G. Birch and K.J. Parker (editors), Intermediate Moisture Foods. Applied Science, London, pp. 120–137.

Nagao, M., Sata, S. and Sugimura, T. (1983) Mutagens produced by heating foods. ACS Soc. Symp. Ser. 215, 521–526.

Pitt, J.I. (1975) Xerophilic fungi and the spoilage of foods of plant origin. In: R.B. Duckworth (editor), Water Relations of Foods. Academic Press, London, pp. 273–307.

Pitt, J.I. and Hocking, A.D. (1985) Fungi and food spoilage. Academic Press, Sydney.

Pitt, J.I., Hocking, A.D., Samson, R.A. and King, A.D. (1992) Recommended methods for the mycological examination of foods, 1992. In: R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King (editors), Modern Methods in Food Mycology. Elsevier, Amsterdam, pp. 365–368.

Samson, R.A., Hocking, A.D., Pitt, J.I. and King, A.D. (1992) Modern Methods in Food Mycology. Elsevier, Amsterdam, 388 pp.

Scarr, M.P. and Rose, D. (1965) Assimiliation and fermentation patterns of osmophilic yeasts in sugar broths at two concentrations. Nature, London 207, 887.

Shibamoto, T. (1983) Heterocyclic compounds in browning and browning-nitrite model systems: occurrence, formation mechanisms, flavour characteristics, and mutagenic activity. In: G. Charalambous (editor), Instrumental Analysis of Foods, Vol. 1. Academic Press, New York, pp. 229–278.

Tilbury, R.H. (1980) Xerotolerant (osmophilic) yeasts. In: S.M. Passmore and R.R. Davenport (editors), Soc. Appl. Bacteriol. Symp. Ser. 9, F.A. Skinner, Biology and Activities of Yeasts. Academic Press, London, pp. 153–179.