J. Gen. Appl. Microbiol., **37**, 111–119 (1991)

MINIMUM WATER ACTIVITIES FOR THE GROWTH OF YEASTS ISOLATED FROM HIGH-SUGAR FOODS

KEIKO TOKUOKA* AND TAKASUKE ISHITANI†

National Food Research Institute, 2–1–2 Kannondai, Tsukuba, Ibaraki 305, Japan

(Received December 4, 1990)

Minimum water activities (a_w) for the growth of 35 yeast strains isolated from high-sugar foods and related materials were determined by incubating in broths containing 1% glucose, 0.5% polypeptone, 0.3% yeast ext., 0.3% malt ext. and a_w-controlling solutes at 25°C for up to 120 days. Three kinds of sugars (glucose, fructose, sucrose) and sodium chloride were used as a_w-controlling solutes. The minimum a_w for growth of the yeasts depended on a_w-controlling solutes as well as yeast species and strains. Most species showed the highest minimum a_w for growth in NaCl-media and about half of the species showed the lowest minimum a_w for growth in sucrose-media. One strain of *Zygosaccharomyces rouxii* had minimum a_w for growth as low as 0.67 in fructose-media. Miso ext. and koji ext., rich in inositol, and casamino acid decreased minimum a_w for growth of *Z. rouxii* in NaCl-media. Pre-incubation in the presence of high concentrations of glucose or fructose also decreased minimum a_w for growth of yeasts in glucose-media or fructose-media.

Water activity (a_w) is one of the important environmental factors affecting microbial growth and the minimum a_w for growth are useful indicators in food preservation. In previous studies (*1, 2, 3, 7*), many yeasts grew at a_w higher than 0.88, while osmophilic or osmotolerant yeasts grew at lower a_w levels. Some strains of the genus *Zygosaccharomyces*, typical osmophilic yeasts, were reported to tolerate an a_w as low as 0.65 (*6, 14*), and the lowest minimum a_w for growth recorded at present is 0.62 (*13*). Although the water relations of the genus *Zygosaccharomyces* were previously studied, there are few papers describing the minimum a_w for the growth of sugar-tolerant yeasts, which belong to many genera and species as reported (*15*).

* Address reprint requests to: Ms. Keiko Tokuoka, National Food Research Institute, 2–1–2, Kannondai, Tsukuba, Ibaraki 305, Japan.

† Present Address: Agricultural Research Center, 3–1–1, Kannondai, Tsukuba, Ibaraki 305, Japan.

We determined the minimum a_w for the growth of yeasts isolated from many foods containing high concentrations of sugar, using four kinds of solutes. It has been found that they depend on the solutes for controlling a_w and other environmental factors as well as yeast species and strains.

MATERIALS AND METHODS

Strains. Among the strains isolated from a variety of high-sugar foods (*15*, *16*), 35 yeast strains belonging to 31 species of 12 genera were tested. Five of those strains belonged to *Zygosaccharomyces rouxii*, a representative osmophilic yeast. All the strains are stored in the National Food Research Institute.

Preparation of media and measurement of a_w. YM agar slant and two kinds of agar slants containing 50% (w/w) glucose or 40% (w/w) fructose, 0.5% polypeptone, 0.3% yeast ext., and 0.3% malt ext. were prepared for pre-incubation. Three ml of broths containing 1% glucose, 0.5% polypeptone, 0.3% yeast ext., 0.3% malt ext., and different percentages of solutes for controlling a_w(a_w-controlling solutes) were prepared in 7-ml test tubes. The test tubes were tightly sealed with screw-caps to prevent changes in the a_w of the broths. Glucose, fructose, sucrose, and sodium chloride were used as a_w-controlling solutes. Three other kinds of broth were also used to prepare media rich in sodium chloride; Medium A: 90 ml of YM broth and 10 ml of miso extract, Medium B: 90 ml of koji extract, 10 ml of raw soy sauce, and 0.5% glucose, Medium C: 5% glucose, 1% casamino acid, 0.2% yeast ext., 0.2% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$.

The a_w of the media were measured at 25°C using an electric hygrometer (Hygroskop DT (Rotronic, Switzerland)).

Incubation. All the strains were pre-incubated on YM agar medium for 2–3 days. The yeast cells were suspended in sterilized 0.87% sodium chloride solution and inoculated into broths with different a_w. The initial cell concentration was 10^2/ml. The inoculated broths were incubated at 25°C for up to 120 days. During incubation, the growth of yeasts was examined by observing the turbidity of broths and counting yeast cells by the plating method.

Five strains of *Z. rouxii* pre-incubated on YM agar were also incubated in Medium A, Medium B, and Medium C to study the effects of nutrients in media.

Several strains were pre-incubated on agar media containing 50% (w/w) glucose or 40% (w/w) fructose, and incubated in the same ways to examine the effects of pre-incubation in the presence of high concentrations of a_w-controlling solutes.

RESULTS

The relationships between the concentrations of four kinds of solutes (expressed as g per 100 g solutions) and the a_w of media containing these solutes are shown in Fig. 1. Sodium chloride was the most effective solute for decreasing a_w because of its small molecular weight and dissociation. On the other hand, sucrose was the

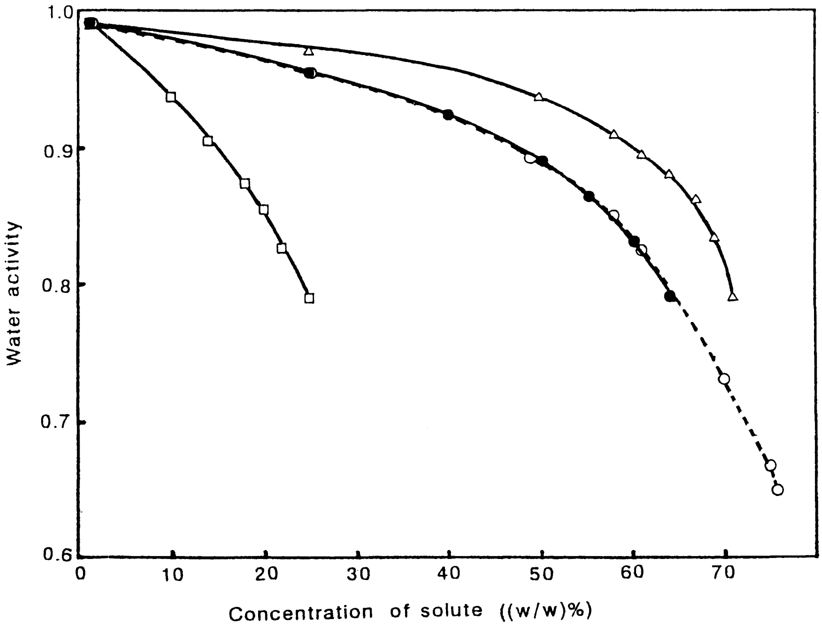

Fig. 1. Relationships between the a_w of media and the concentrations of a_w-controlling solutes.

Media contained 1% glucose, 0.5% polypeptone, 0.3% yeast ext., 0.3% malt ext., and different concentrations of a_w-controlling solutes.

●: glucose-media, ○: fructose-media, △: sucrose-media, □: NaCl-media.

least effective.

Glucose and sucrose have lower solubility in water than fructose and the lowest a_w of broth to which they could adjust was 0.79 (64% (w/w) glucose or 71% (w/w) sucrose), while fructose could adjust the a_w of broth to a level as low as 0.65 (76% (w/w) fructose).

Table 1 shows the minimum a_w for growth of 35 strains of yeasts isolated from high-sugar foods. They differed significantly from the species to species. There were also differences between the minimum a_w for growth of five strains of *Z. rouxii.*

The effects of a_w-controlling solutes on the minimum a_w for the growth of each strain were observed. In most species, incubation in broths containing sodium chloride as the a_w-controlling solute (NaCl-media) gave higher minimum a_w for growth than those in broths containing glucose, fructose, or sucrose (glucose-, fructose-, or sucrose-media). Only a few species, such as *Debaryomyces hansenii* and *Hansenula anomala,* showed similar minimum a_w for growth in NaCl-media and three kinds of sugar-media.

The effects of three kinds of sugars on minimum a_w for growth differed from species to species. About half of the species showed the lowest minimum a_w for growth in sucrose-media and about one third showed the lowest values in

Table 1. Some characteristics and minimum a_w for growth of

Strain		Source	Assimilation	
			Glucose	Fructose
Debaryomyces hansenii	67-40A	blueberry sauce	+	+
Hansenula anomala	150-40A	sponge cake	+	+
Pichia ohmeri	19-40A	flower	+	+
Pichia membranaefaciens	114-CA	soil	+	+
Saccharomyces cerevisiae	89-25A	candied apple	+	+
Schizosaccharomyces pombe	57-50B	molasses	+	+
Torulaspora delbrueckii	154-CA	sweet bean paste	+	+
Torulaspora globosa	26-40A	papaya	+	+
Zygosaccharomyces bisporus	153-40A	molasses	+	+
Zygosaccharomyces rouxii	38-40A	honey	+	+
Zygosaccharomyces rouxii	86-A	soy sauce	+	+
Zygosaccharomyces rouxii	87-A	miso	+	+
Zygosaccharomyces rouxii	88-40A	candied cherry	+	+
Zygosaccharomyces rouxii	151-40A	molasses	+	+
Candida apicola	22-40A	apple	+	+
Candida bombi	19-25A	flower	+	+
Candida bombicola	34-40B	flower	+	+
Candida dattila	128-25A	raisin	+	+
Candida dulciaminis	155-CA	sponge cake	+	+
Candida famata	147-40A	sap	+	+
Candida glucosophila	29-25A	brown sugar	+	+
Candida guilliermondii	22-25A	apple	+	+
Candida intermedia	147-CA	sap	+	+
Candida lactiscondensi	91-40A	angelica	+	+
Candida lusitaniae	91-CA	angelica	+	+
Candida mannitofaciens	152-40A	molasses	+	+
Candida nodaensis	24-60A	kiwi fruit	+	+
Candida oregonensis	107-40A	flower	+	+
Candida tropicalis	118-40A	lemon cake	+	+
Candida versatilis	24-60B	kiwi fruit	+	+
Kloeckera apis	26-50A	papaya	+	+
Rhodotorula glutinis	13-40A	flower	+	+
Rhodotorula rubra	70-25A	jam	+	+
Rhodosporidium sp.	65-25A	dried date	+	+
Sympodiomycopsis paphiopedili	101-40A	nectar	+	+

+: positive, −: negative, w: weak.

glucose-media and sucrose-media.

The strain which showed the lowest a_w for growth (0.67) was *Z. rouxii* 38-40A. This was shown in fructose-media. *Zygosaccharomyces bisporus*, *Candida apicola*, *Candida bombi*, *Candida glucosophila*, *Candida lactiscondensi*, *Candida mannitofaciens*, *Candida nodaensis* and *Candida versatilis* also had minimum a_w for growth lower than 0.79.

Table 2 shows the minimum a_w for the growth of five strains of *Z. rouxii* in yeasts isolated from high-sugar foods and related materials.

Assimilation	Minimum a_w for growth in			
Sucrose	Glucose-media	Fructose-media	Sucrose-media	NaCl-media
+	0.84	0.86	0.81	0.84
+	0.86	0.91	0.84	0.84
+	0.84	0.86	0.83	0.90
−	0.90	0.92	0.90	0.94
+	0.89	0.91	0.89	0.92
+	0.84	0.86	0.87	0.95
+	0.86	0.89	0.87	0.90
+	0.86	0.89	0.85	0.90
−	0.85	0.85	≦0.79	0.95
−	≦0.79	0.67	≦0.79	0.95
−	≦0.79	0.73	≦0.79	0.92
−	≦0.79	0.83	≦0.79	0.95
−	0.81	0.82	≦0.79	0.88
w	≦0.79	0.82	≦0.79	0.86
+	≦0.79	0.79	≦0.79	0.90
+	≦0.79	0.83	≦0.79	0.90
+	0.84	0.85	0.85	0.92
+	0.88	0.91	0.87	0.90
+	0.84	0.91	0.81	0.86
+	0.91	0.92	0.90	0.94
−	0.81	0.85	≦0.79	0.83
+	0.89	0.89	0.85	0.88
+	0.90	0.92	0.89	0.92
+	≦0.79	0.78	≦0.79	0.92
+	0.90	0.92	0.87	0.90
+	≦0.79	0.73	≦0.79	0.86
−	0.81	0.80	≦0.79	0.82
+	0.86	0.89	0.85	0.90
+	0.88	0.88	0.83	0.90
+	≦0.79	0.80	≦0.79	0.84
−	0.90	0.93	0.90	0.95
−	0.89	0.93	0.89	0.92
+	0.90	0.92	0.90	0.90
+	0.89	0.91	0.89	0.94
+	0.89	0.91	0.85	0.90

four kinds of media. The minimum a_w for the growth of the two strains 88-40A and 151-40A, isolated from high-sugar foods, significantly decreased in Media A, B, and C. Medium B was particularly effective. But the effects on the a_w of strain 38-40A were small. The minimum a_w for the growth of the two strains 86-A and 87-A, isolated from soy sauce and miso, respectively, decreased only in Media A and C.

Tables 3 and 4 show the effects of pre-incubation in the presence of high

Table 2. Comparison of minimum a_w for growth of *Zygosaccharomyces rouxii* in four kinds of NaCl-media.

Strain	Minimum a_w for growth in			
	YM+NaCl	Medium A	Medium B	Medium C
Zygosaccharomyces rouxii 38-40A	0.95	0.95	0.93	0.94
Zygosaccharomyces rouxii 86-A	0.88	0.86	0.88	0.85
Zygosaccharomyces rouxii 87-A	0.86	0.82	0.86	0.85
Zygosaccharomyces rouxii 88-40A	0.92	0.86	0.84	0.89
Zygosaccharomyces rouxii 151-40A	0.95	0.92	0.86	0.89

Medium A: 90 ml of YM broth, 10 ml of miso ext., and NaCl.
Medium B: 90 ml of koji ext., 10 ml of raw soy sauce, 0.5% glucose, and NaCl.
Medium C: 5% glucose, 1% casamino acid, 0.2% yeast ext., 0.2% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and NaCl.

Table 3. Effects of pre-incubation in the presence of high concentrations of glucose on minimum a_w for growth of yeasts.

Strain		Pre-incubation on	
		YM	50% (w/w) glucose-medium
Debaryomyces hansenii	67-40A	0.86	0.80
Torulaspora delbrueckii	154-CA	0.86	0.80
Zygosaccharomyces rouxii	38-40A	0.85	0.80
Candida bombicola	34-40B	0.86	0.80
Candida nodaensis	24-60A	0.83	0.75

Table 4. Effects of pre-incubation in the presence of high concentrations of fructose on minimum a_w for the growth of yeasts.

Strain		Pre-incubation on	
		YM	40% (w/w) fructose-medium
Hansenula anomala	150-40A	0.89	0.85
Torulaspora delbrueckii	154-CA	0.90	0.85
Candida dattila	128-25A	0.91	0.86
Candida lusitaniae	91-CA	0.91	0.87
Candida oregonensis	107-40A	0.91	0.87
Rhodotorula glutinis	13-40A	0.91	0.87

concentrations of sugars on the minimum a_w for the growth of several strains. Pre-incubation on agar media containing 50% (w/w) glucose or 40% (w/w) fructose decreased the minimum a_w for growth in glucose-media or fructose-media of all strains examined.

DISCUSSION

The results in Fig. 1 agree with the previous reports. Kushner (*5*) showed the relationship between the concentrations of different solutes (expressed as percent (w/v)) and a_w of these solutions. Since the concentrations of solutes were expressed as percent (w/w) in this study, the dependences of a_w on solute concentrations were more drastic.

Several species belonging to the genus *Candida* such as *Candida apicola* and *Candida bombicola* showed low minimum a_w for growth in sugar-media, although most of previous papers described low minimum a_w for the growth of only the genus *Zygosaccharomyces*.

Three strains of *Z. rouxii* isolated from high-sugar foods were only sugar-tolerant, while two strains of *Z. rouxii* isolated from high-salt foods such as soy sauce and miso were both sugar-tolerant and salt-tolerant. These results agree with the report by Onishi (*11*) that the salt-tolerance and sugar-tolerance of *Z. rouxii* differ considerably, strain by strain, according to their origin. The fact that most of the yeast strains isolated from high-sugar foods showed lower minimum a_w for growth in sugar-media compared with NaCl-media indicated that the mechanism of sugar-tolerance differs from that of salt-tolerance. Onishi (*10*) found that the differences between sugar-tolerance and salt-tolerance were also observed in pH ranges for growth. Inhibition of microbial growth by sodium chloride could be caused by inhibition of enzyme activities, decrease of solubilites of oxygen, increased sensitivities of cells to carbon dioxide, or the toxic action of chrome ions, and so on.

One factor in the differences of minimum a_w for growth in the presence of different sugars could be a difference between the mechanism of glucose tolerance and that of sucrose tolerance. Moran and Witter (*8*) found that production of D-arabitol increased when *Z. rouxii* (*Saccharomyces rouxii*) cells were grown in the presence of increasing glucose concentrations, while growth in high concentrations of sucrose had no effect on the production of D-arabitol. Another factor could be a difference between the protective action of sugars on yeast cells. Sucrose was likely to have the largest action to protect yeast cells among three kinds of sugars, because yeast cells were alive even below minimum a_w for growth in sucrose-media, although they died out below the minimum a_w for growth in glucose-media and fructose-media. This action of sucrose decreased the minimum a_w for growth in the strains which did not assimilate sucrose as well as in the strains which did assimilate it. This shows that the ability or inability of yeasts to utilize carbon compounds does not always contribute to such decreases of minimum a_w for growth.

The minimum a_w for growth are affected by other environmental factors such as temperature, pH, concentration of oxygen and carbon dioxide, and nutrients; they are the lowest when these factors are most suitable for microbial growth. This study was carried out under almost the most suitable conditions. Sato et al. (*12*) and Onishi (*10*) reported that *Z. rouxii* required vitamins such as inositol, panthotenic acid and biotin in the presence of sodium chloride. Kato et al. (*4*) found that soy sauce markedly accerelated the growth of osmophilic yeasts and identified the effective component as myo-inositol. We examined the effects of miso ext. and koji ext. containing raw soy sauce, which were rich in inositol, and casamino acid as a nitrogen source on the minimum a_w for the growth of *Z. rouxii*. Results in Table 2 support the previous papers describing acceleration of growth by inositol and demonstrate that casamino acid also decreased minimum a_w for growth.

The fact that pre-incubation in the presence of high concentrations of sugars decreased the minimum a_w for growth showed that the osmotolerance of the yeast cells were affected by adaptation to environment, although this is generally considered to be genetic. This agreed with a previous paper describing the decrease in survival of cells by transporting cells to media containing considerablly different concentrations of sodium chloride (*9*).

Some strains tested in this study had low minimum a_w for growth below 0.80. This suggests that combinations of low a_w with other environmental factors such as lower temperatures and lower concentrations of oxygen are important for preventing spoilage by yeasts in foods with intermediate moisture.

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