

Microbiological Quality, Shelf Life and Fermentation Activity of Active Dried Yeast¹⁾

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The microbiological quality and shelf life of 10 active dried yeast preparations were determined. The dried yeasts were tested for total microbial count, total yeast count, total wild yeast count, total bacterial count, spore forming bacteria, Enterobacteriaceae and lactic acid bacteria. For the shelf life test, the yeasts were stored at 0°C, 4°C, 15°C and 25°C for six months and tested at monthly intervals for total viable yeast count and total bacterial count. After six months storage fermentations were carried out in Colombar must and the wines subsequently analysed. All dried yeasts complied with the International Wine Office (O.I.V.) guideline of 1×10^9 viable yeasts per gram dried yeast, whilst only some preparations complied with the guideline for total bacterial count of 1×10^5 bacteria per gram dried yeast. Results of the shelf life test showed optimum storage temperature to be 4°C.

Cuinier & Lacoste (1980) stated that the use of active dried yeast preparations (ADYP) presents the following advantages when compared to wines having undergone spontaneous fermentation: decrease in the lag phase, better completion of alcoholic fermentation, less acetaldehyde formed in certain wines and the absence of organoleptic differences.

The use of ADYP, especially in countries such as South Africa and Australia has, to a large extent, replaced spontaneous fermentations (Rankine, 1978). While various authors (Bauer & Klein Henz, 1978; Rankine, 1978; Cuinier & Lacoste, 1980; Bidan & Maugenet, 1981) noted that wines prepared with ADYP did not differ from wines inoculated with liquid starters and spontaneous fermentations. On the other hand, Gaia & Gandini (1983) found that wines inoculated with ADYP were superior to spontaneously fermented wines.

Radler (1978) proposed the following criteria for ADYP quality: the total number of yeasts, the viability, the level of contaminating micro-organisms, indigenous yeasts, moulds, bacteria, lactic acid bacteria, the presence of additives and the date of production. Present guide lines of the International Wine Office (O.I.V.) in Paris, France, stipulate a minimum of 1×10^9 viable yeasts per gram ADYP and a maximum of 1×10^5 bacteria per gram ADYP (Anon., 1981). It is clear that the existing guidelines for the quality of ADYP are insufficient.

ADYP have a shelf life of one to three months when exposed to air or as long as one year when packed under nitrogen or vacuum (Chen, Cooper & Gutmanis, 1966; Reed & Chen, 1978). Packaging under nitrogen or vacuum does, however, increase the cost of ADYP. They further note that shelf life, even with different packaging, is dependent on storage temperature.

The purpose of this study was to determine the microbiological quality of ADYP, especially wine yeasts, from different manufacturers with a view to possibly establishing guidelines for ADYP quality. The

effect of storage temperature on cell viability and fermentation ability was also examined.

MATERIALS AND METHODS

Yeast strains:

All ADYP included in this study were *Saccharomyces cerevisiae* strains. Six locally produced ADYP viz. WE 14, WE 372 (batches 1 and 2), WE 500 (batches 1 and 2) and a bakers' yeast strain 342, were obtained directly from the producer. Hefix 1000, V-1116, Siha-I and M-1107 are imported ADYP and were obtained from a local winery.

Microbiological quality:

The microbiological quality of each batch of ADYP was determined immediately after receipt. Tests and media used, were the following: total microbial count – plate count agar (Oxoid); total viable yeasts – Yeast Morphology (YM) agar (Difco); total wild yeasts – Lysine medium (Oxoid); total bacterial count – plate count agar (Oxoid); spore forming bacteria – plate count agar (Oxoid); Enterobacteriaceae – violet red bile agar (Merck); lactic acid bacteria – MRS agar (Merck) and tomato juice agar (Difco). For the bacterial tests Actidione (BDH) (0,1 g/l) was added to the media to suppress yeast growth. The pH of the medium used for yeast counts was lowered to pH 5,0 to suppress bacterial growth and all determinations were done in triplicate.

Rehydration of the ADYP was carried out in physiological salt solution (PSS) in test tubes in a waterbath at 43°C for 30 minutes. Dilution series were made in PSS and plated out in triplicate.

The presence of spore forming organisms was determined by placing the rehydrated ADYP in a waterbath at 80°C for 10 minutes. Plates were incubated at 30°C, except the violet red bile agar plates which were incubated at 37°C. Plate counts were done after three days with the exception of the Lysine medium which was examined on days three and five and counted on day seven. The lactic acid bacterial counts were done after

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one week. Yeast and bacterial counts are expressed per gram ADYP.

Shelf life:

The shelf life of the different ADYP was determined by storing samples of each strain at 0°C, 4°C, 15°C and 25°C for six months and doing a total viable yeast count, a total bacterial count and, where necessary, a wild yeast count at monthly intervals.

The monthly counts were carried out as described under microbiological quality. Results were expressed as percentage viable cells with the original count as 100%.

Accelerated shelf life test:

After receiving the ADYP, an accelerated shelf life test was carried out by placing samples of the ADYP in air tight containers at 45°C for 28 days. Dilution plate counts for viable yeast cells were made at regular intervals for up to 28 days on YM agar. Results were expressed as percentage surviving cells taking the cell count of day 0 as 100%.

Fermentation activity:

Fermentation activities were determined on all the samples of ADYP stored at the various temperatures. One gram dried yeast was rehydrated in 10 ml sterilised water and 1,5 ml of this suspension was used to inoculate triplicate lots of 500 ml Colombar must. The must

had a sugar content of 160 g/l, a total acidity of 5,4 mg/l, a pH of 3,59, a total SO₂ content of 29 mg/l and a free SO₂ content of 5 mg/l. The fermentation progress at 15°C was followed by determining CO₂ loss through regular weighing. After 21 days the fermentations were stopped and the wines were analysed for alcohol, sugar, volatile acidity, total acidity and pH, using standard Viticultural and Oenological Research Institute (V.O.R.I.) methods.

RESULTS AND DISCUSSION

Microbiological quality:

Results of the microbiological tests carried out on the ADYP are presented in Table 1. The total microbial count varied from 8,2 x 10⁹ for WE 14 to 5,9 x 10¹⁰ for WE 372 (batch 2) and the total viable yeast counts varied from 1,3 x 10⁹ (Hefix 1000) to 6,1 x 10¹⁰ for WE 372 (batch 2). All the ADYP included in this study therefore complied with the guideline of 1 x 10⁹ viable yeast cells/g ADYP set by the O.I.V. (Anon., 1981). Bidan & Maugenet (1981) noted that a total of 38 ADYP, studied by various authors, were found to have viable yeast counts varying from 1 x 10⁹ to 5 x 10¹⁰. Results obtained in this study confirm these findings. The present guideline for a total viable yeast count of 1 x 10⁹/g ADYP as laid down by the O.I.V., is regarded as a realistic figure and should be accepted.

TABLE 1.

Microbial counts per gram active dried yeast preparation (ADYP)

Microbial Tests	ADYP									
	WE 372 Batch 1	WE 372 Batch 2	WE 14	WE 500 Batch 1	WE 500 Batch 2	Bakers' Yeast	Hefix 1000	V-1116	Siha-I	M-1107
Total microbial count	2,3x10 ¹⁰	5,9x10 ¹⁰	8,2x10 ⁹	1,1x10 ¹⁰	1,2x10 ¹⁰	1,6x10 ¹⁰	3,6x10 ¹⁰	4,4x10 ¹⁰	2,1x10 ¹⁰	1,3x10 ¹⁰
Total yeast count	2,8x10 ¹⁰	6,1x10 ¹⁰	8,0x10 ¹⁰	1,2x10 ¹⁰	1,3x10 ¹⁰	1,6x10 ¹⁰	1,3x10 ⁹	2,4x10 ¹⁰	2,5x10 ¹⁰	2,0x10 ⁹
Wild yeast count	0	1,0x10 ²	0	6,6x10 ⁶	8,5x10 ³	8,5x10 ⁶	2,0x10 ²	4,2x10 ⁴	0	0
Total bacterial count	9,0x10 ⁴	3,1x10 ⁶	1,2x10 ⁵	4,7x10 ⁶	4,1x10 ⁷	4,4x10 ⁷	4,4x10 ⁸	2,5x10 ⁵	1,0x10 ⁴	1,9x10 ⁵
Spore forming bacteria	3,7x10 ²	4,0x10 ²	6,7x10 ²	1,3x10 ²	4,8x10 ²	1,0x10 ²	2,7x10 ²	6,0x10 ²	0	8,0x10 ²
Enterobacteriaceae	0	1,0x10 ²	0	1,2x10 ⁵	7,1x10 ⁵	1,6x10 ⁵	1,3x10 ²	2,0x10 ³	6,7x10 ²	1,3x10 ²
Lactic acid bacteria (MRS agar)	1,9x10 ³	3,2x10 ⁶	1,0x10 ⁵	3,0x10 ⁶	2,8x10 ⁶	7,1x10 ⁷	3,8x10 ⁸	1,8x10 ⁵	2,0x10 ⁴	8,0x10 ⁴
Lactic acid bacteria (Tomato juice agar)	4,0x10 ⁴	2,8x10 ⁶	8,0x10 ⁴	5,0x10 ⁶	1,1x10 ⁶	1,4x10 ⁸	4,5x10 ⁸	1,9x10 ⁵	6,0x10 ³	3,0x10 ⁴

Large variation was found in the wild yeast counts. Four ADYP were found to contain no wild yeasts, whilst the wild yeast counts for the remaining ADYP varied from 1,0 x 10² to 8,5 x 10⁶. At present no guideline has been set by the O.I.V. for wild yeast counts in ADYP. It was commonly accepted that wild yeasts, especially of the genera *Kloeckera*, *Hansensiaspora*, *Candida*, *Pichia* and sometimes *Hansenula*, grow during the early stages of must fermentation but eventually die off (Fleet, Lafon-Lafourcade & Ribéreau-Gayon, 1984; Lafon-Lafourcade & Ribéreau-Gayon, 1984). Benda (1981) and Fleet *et al.* (1984) state that these genera may have important effects on wine flavor. However, Heard & Fleet (1985) have shown that certain wild yeasts notably *Kloeckera apiculata*, *Candida stellata* and *C. pulcherrima* could contribute to wine fermentation. Care should be taken in using ADYP with too high a wild yeast count since *Sacch. cerevisiae* ADYP do not suppress the development of all natural yeasts during wine fermentation (Heard & Fleet, 1985).

A maximum of 1 x 10³ wild yeasts/g ADYP should be an acceptable guideline.

The total bacterial counts varied between ADYP with Siha-I containing the least bacteria (1 x 10⁴), whilst Hefix 1000 had the highest total bacterial count (4,4 x 10⁸). The high count for Hefix 1000 can possibly be ascribed to the high number of lactic acid bacteria (see later). Siha-I was the only ADYP that did not contain any spore forming bacteria, whilst all the other preparations contained relatively low amounts of spore forming bacteria (between 1 x 10² and 8 x 10²). Only WE 372 (batch 1) and WE 14 ADYP did not contain any Enterobacteriaceae whilst the concentration in the other ADYP studied, varied from 1,3 x 10² for M-1107 to 7,1 x 10⁵ for WE 500 (batch 2) (Table 1). Benda (1984) found that even large numbers (10⁷ cells per ml) of coliform did not survive longer than 48 hours in the wines that were tested. It should however be noted that the pH of wines studied by Benda (1984) was lower than 3,6 and the free SO₂ concentration higher than

33 mg/l. It is possible that Enterobacteriaceae will survive for longer periods, should these conditions change, especially where the wine pH is notably higher (eg. pH 4).

O.I.V. guidelines for ADYP state that the bacterial counts should not exceed 1×10^5 bacteria/g ADYP and that the test be carried out on MRS agar which is a selective medium for Lactobacilli (Man, Rogosa & Sharp, 1960). This could, however, lead to misinterpretation of the total bacterial count for the following reasons:

1. MRS agar is a selective medium for lactic acid bacteria, therefore other organisms present will not easily be detected;
2. and organisms of the species *Leuconostoc oenos* have a specific requirement for a tomato juice growth factor (Garvie & Mabbitt, 1967) and will therefore not grow on MRS agar.

This study has shown that on average a higher recovery of lactic acid bacteria was obtained on tomato juice agar than on MRS agar (Table 1). The differences between the individual counts on the tomato juice agar and MRS agar can be ascribed to the differences in the lactic acid bacteria populations of the various ADYP. Furthermore, the total bacterial count was either of the same order or higher than the counts for lactic acid bacteria, with the exception of the bakers' yeast. It is therefore recommended that both a total bacterial count on a medium such as plate count agar and a lactic acid bacteria count on tomato juice agar be carried out on ADYP.

Only four of the ADYP tested (WE 372 (batch 1), WE 14, Siha-I and M-1107) complied with the O.I.V. guidelines for bacterial counts, whilst the lactic acid bacteria counts for the other ADYP were notably higher, up to $4,5 \times 10^8$. Radler, Dietrich & Schönig (1985) found that the six ADYP studied by them, contained up to 2×10^7 bacteria. Tromp & Strydom (1980), using tomato juice agar, found that the lactic acid bacteria count for six ADYP varied from 1×10^4 to 1×10^7 . However, the present work has shown that the counts on the same medium for the ADYP studied varied from 6×10^3 to $4,5 \times 10^8$ (Table 1). A total bacterial count of 5×10^6 /g ADYP and a lactic acid bacteria count of 1×10^6 /g ADYP should be the accepted norms for these tests.

Shelf life:

The percentage surviving yeast cells after six months at the various storage temperatures is presented in Table 2. Of the two batches of strain WE 372 tested, batch 1 had a much better shelf life than batch 2.

Batch 1 of this strain showed a better shelf life at 0°C, 4°C and 15°C (above 81%), than batch 2 which had only 62% viable yeasts after six months at 0°C and markedly lower counts at the other temperatures. This shows that shelf life is not a characteristic of a specific yeast strain, but depends on manufacturing conditions.

Over the six months period the viable yeast counts for strain WE 14 were 66% and 69% at 0°C and 4°C respectively, which were markedly higher than at the higher storage temperatures. Both batches of WE 500 showed tendencies similar to WE 14 at 0°C and 4°C whilst at 25°C the viable yeast count for WE 500 was markedly lower. The shelf life of the bakers' yeast strain was comparable to WE 500 although the keeping

TABLE 2

Percentage survival of total viable yeasts of active dried yeast preparations stored at various temperatures for six months. (Values in Table 1 taken as 100%)

Yeast strain	Survival (%)			
	0°C	4°C	15°C	25°C
WE 372 (Batch 1)	84,0	100,0	81,0	34,0
(Batch 2)	62,0	33,0	31,0	15,0
WE 14	66,0	69,0	45,0	23,0
WE 500 (Batch 1)	74,0	63,0	28,0	0,1
(Batch 2)	55,0	61,0	47,0	4,0
Bakers' yeast 343	70,0	71,0	50,0	2,0
Hefix 1000	40,0	35,0	1,0	3,0
V-1116	96,0	98,0	96,0	63,0
Siha-I	60,0	56,0	50,0	12,0
M-1107	72,0	70,0	62,0	55,0

quality was better at 0°C and 4°C. Of all the strains tested Hefix 1000 had the poorest shelf life with only 40% and 35% survival at 0°C and 4°C respectively after six months.

Strain V-1116 had the best keeping quality at all temperatures of all strains studied, especially at 15°C and lower. The shelf life at 0°C, 4°C and 15°C of Siha-I varied from 50 to 60% whilst only 12% surviving yeasts were counted at 25°C after six months. With the exception of 25°C, M-1107 yielded results similar to those of the bakers' yeast strain. Tromp & Strydom (1980) recommended that storage should be below 20°C. Results of this study indicate that storage should preferably be carried out at 4°C. Little variation was obtained between 0°C and 4°C.

Accelerated shelf life test:

An accelerated shelf life test was previously done at the V.O.R.I. on the ADYP at 45°C for 21 days. This test was included in this study to determine whether there is any relationship between the accelerated shelf life test and the keeping quality at the different storage temperatures. However, no relationship was found at 0°C, 4°C and 15°C, whilst in some cases similar results were obtained with the accelerated shelf life test and storage at 25°C. This fact is substantiated by, for example, strain WE 372 (batch 1) which had, on average, a 88% survival after six months at 0°C, 4°C and 15°C, whilst with the accelerated shelf life test only 9% viable yeasts were counted after 28 days. Although there was only a slight loss of viability for strain V-1116 at 0°C, 4°C and 15°C after six months, the accelerated shelf life test showed only 25% viability after five days and 2,5% after 28 days. The accelerated shelf life test, as included in this study, is therefore not effective for testing the shelf life of ADYP and a substitute test should be found.

Fermentation activity:

Yeast stored at higher temperatures had longer lag phases and slower fermentation rates than those stored at lower temperatures (Fig. 1). The longer lag phase and slower fermentation rate can be ascribed to the loss of viability at the higher temperatures. However, three ADYP (WE 372 (batch 1), WE 500 (batch 2) and V-1116) stored at 15°C displayed similar fermentation curves to those stored at 0°C and 4°C. Strain V-1116 which had the best shelf life was the only strain where the fermentation activity of the ADYP stored at 25°C was comparable with the results obtained at the other tempera-

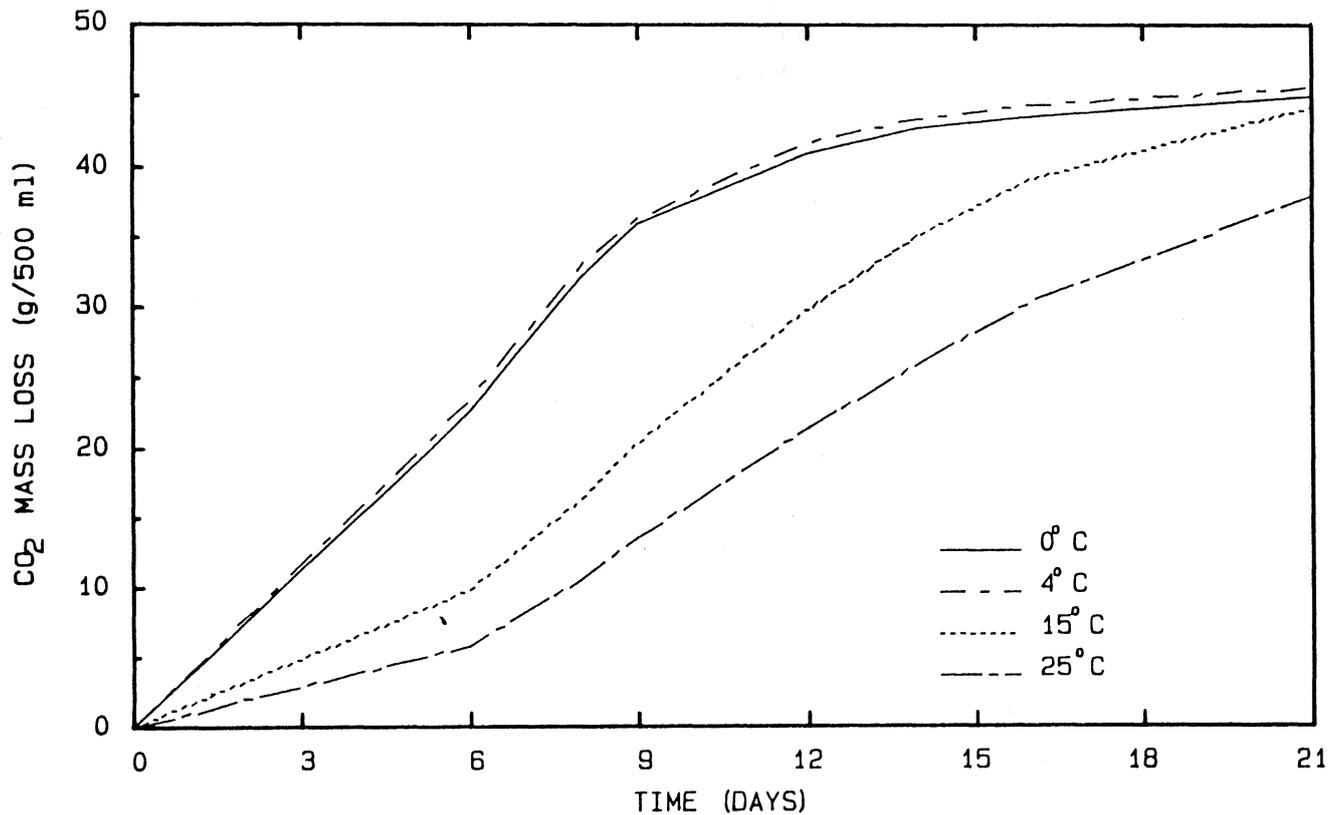


FIG. 1

Fermentation activity of WE 500 in Colombar must after storage for six months at different temperatures.

tures. Previously it has been shown that reserve carbohydrates (trehalose and glycogen) effect the shelf life and fermentation activity of ADYP (Bocharova, Chernysh & Ozerova, 1973). It is possible that differences in reserve carbohydrates may be one of the reasons for the above differences.

Wine analyses:

Of all the analyses carried out on the wines, only the pH remained constant (data not shown). From Table 3 it is clear that the higher the ADYP storage temperature, the higher the residual sugar concentration and thus the lower the alcohol concentration. This is true for all the ADYP with the notable exceptions of WE 372 (batch 2) and V-1116. The volatile acidity increases with an increase in storage temperature, possibly due to the longer lag phases and slower fermentation rates of

these fermentations. This is a common phenomenon which has not yet been explained.

As was the case with the viable yeast cell counts after storage at the different temperatures for six months, strains WE 372 (batch 2) and V-1116 did not show any notable difference in the analyses of the wines. Both batches of WE 500 and strain M-1107 showed large differences in shelf life at the different storage temperatures. Likewise the analyses of the wines made from these strains showed large differences. Total acidity of the wines showed similar tendencies to the volatile acidity.

CONCLUSIONS

On comparison of the microbiological quality of the ADYP, it was noted that the total microbial count,

TABLE 3

Sugar concentration and volatile acidity of Colombar wine after fermentation for 21 days with active dried yeast preparations stored at various temperatures for six months.

Yeast strain	Sugar (g/l)				Volatile acid (mg/l)			
	0°C	4°C	15°C	25°C	0°C	4°C	15°C	25°C
WE 372 (Batch 1)	11,9	12,4	11,1	11,5	0,51	0,57	0,72	0,76
(Batch 2)	20,5	11,2	5,3	10,0	0,54	0,44	0,56	0,64
WE 14	4,0	3,8	5,7	15,4	0,58	0,42	0,46	0,64
WE 500 (Batch 1)	6,0	5,0	13,4	35,1	0,55	0,56	1,09	1,18
(Batch 2)	3,6	4,6	4,7	29,8	0,63	0,59	0,73	1,17
Bakers' yeast 342	22,9	23,8	23,9	54,1	0,42	0,40	0,56	0,69
Hefix 1000	34,1	31,7	57,6	63,5	0,43	0,51	0,72	0,72
V-1116	3,1	3,9	2,5	2,9	0,42	0,40	0,42	0,47
Siha-I	7,0	7,7	9,9	12,0	0,40	0,42	0,43	0,57
M-1107	14,8	14,2	18,4	148,3	0,42	0,44	0,55	1,34

total yeast count, total bacterial count and total spore forming bacteria were of the same order, whilst varying concentrations of wild yeasts, Enterobacteriaceae and lactic acid bacteria were found. The six locally produced ADYP yielded similar results for the microbiological tests with the exception of the bakers' yeast strain which contained more lactic acid bacteria and less spore forming bacteria than the other ADYP.

It is recommended that the present guideline for a total viable yeast count of 1×10^9 /g ADYP as laid down by the O.I.V. be accepted. However, a total bacterial

count of 5×10^6 /g ADYP and a lactic acid bacteria count of 1×10^6 /g ADYP should be the accepted norms for these tests. A maximum of 1×10^3 wild yeasts/g ADYP should also be acceptable.

Results clearly indicate that low storage temperatures are essential for the survival of ADYP even for short periods of time. Room temperature (25°C) is clearly not desirable. It must be emphasised that ADYP should be treated with care and stored at low temperatures (0°C to 4°C) even for short periods of time.

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