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EFFECT OF MOLASSES CONCENTRATION AND MEDIUM SUPPLEMENTATION ON THE ADAPTABILITY AND VIABILITY OF A HIGH LEVEL ETHANOL-TOLERANT PALM-WINE *Saccharomyces* ISOLATE*

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SUMMARY

A high level ethanol-tolerant palm wine *Saccharomyces* yeast showed good adaptability (2.6±0.3 – 16.4±0.7 days) in 5–30% unclarified molasses, and good viability in 10–30° Brix molasses-based media at 30°C for 3 days. Media supplementation with soyabean, castor oil bean or groundnut meals improved both yeast adaptability to molasses (X 29–33%) and yeast viability during batch fermentation.

INTRODUCTION

High nutritional quality as well as low costs have made cane molasses a preferred media component for yeast-based ethanologenic processes (Dhamija *et al*, 1986). Unfortunately blackstrap molasses contains high levels of yeast growth – and fermentation – inhibiting substances (Lehtonen and Suomalainen, 1977; Letourneau and Villa, 1987; Essia-Ngang *et al*, 1989). For this reason, it is used either at only very dilute concentrations (12-17% fermentable sugars) (Lehtonen and Suomalainen, 1977; Ake and Bjorling, 1981; Raghav *et al*, 1989) or after clarification i.e. sulphitation and carbonation (Dhamija *et al*, 1986). These in turn make ethanol recovery and production more expensive and difficult (Ake and Bjorling, 1981).

In the assessment of yeast strains for molasses-based ethanologenic processes specific physiological properties are required (Ekunsanmi and Odunfa, 1990). Good tolerance to ethanol, sugar (Benitez *et al*, 1983) and other molasses compounds (Dhamija *et al* 1986; Raghav *et al*, 1989) as well as good invertase activity and an excellent specific ethanol productivity (Stewart, 1985) are top pre-requisites for an efficient process. This paper reports on the ability of a molasses-adapted palm wine *Saccharomyces* isolate to grow in and maintain good viability in 10–30° Brix unclarified molasses based media at 30°C.

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* Dedicated to Dr. A. C. Emeruwa who died before it all started.
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MATERIALS AND METHODS

Yeast strain: The yeast strain used in this study was an ethanol-tolerant *Saccharomyces* yeast (isolate J) from fermenting palm wine juice (Ezeogu and Emeruwa, 1993).

Media: Sugar cane molasses was obtained from the Nigerian Sugar Company Ltd. Medium for yeast adaptation to molasses contained per litre; yeast extract 2g; $(NH_4)_2SO_4$ 4g; $MgSO_4.7H_2O$ 0.7g; KH_2PO_4 1g and molasses adjusted to 5–30° Brix (Table 1). Supplemented adaptation media contained in addition to the above, 35g/l of either whole soyabean, groundnut or castor oil bean meal.

The medium for yeast propagation was MMYP (Patil *et al*, 1989). The medium contained, molasses 5%; malt extract 0.3%; yeast extract 0.3%; and peptone 0.5%. The medium employed for batch fermentation contained the following; $MgSO_4.7H_2O$ 0.7g/l; KH_2PO_4 1g/l; $(NH_4)_2SO_4$ 4g/l, and molasses, adjusted to achieve 10, 15, 20 or 30° Brix as required (Figures 1–5). All supplemented fermentation media contained in addition 35g/l of either soyabean, groundnut or castor oil bean meal. Media were sterilized by autoclaving at 121°C for 15 minutes. Complete supplemented media with the supplements were then filtered aseptically through sterile cotton wool. Media pH was 5.5.

Yeast Adaptation: Yeast cells ($2x10^7$ cells/ml) were inoculated into 50 ml of adaptation media contained in 150 ml cotton wool stoppered Ehrlenmeyer flasks and incubated statically at 30°C. Flasks were then observed 12-hourly until yeasts showed signs of activity (medium effervescence). Supplemented adaptation media were set up to study the effects of supplements on rates of yeast adaptation. Yeast adaptability was measured as the time (days) before yeast activity (medium effervescence) was observed. Enhancement of adaptability was then expressed by the following formula:

$$\% \text{ AE} = \frac{\text{Adaptation value in unsupplemented medium} - \text{Adaptation value in supplemented medium}}{\text{Adaptation value in unsupplemented medium}} \times \frac{100}{1} \quad \ldots 1$$

where AE = Adaptability enhancement

Seed Culture Propagation: Molasses-adapted yeasts from the unsupplemented 30° Brix adaptation medium were grown statically in sterile MMYP medium for 24h at 30°C. After the incubation period, yeast cells were recovered by centrifugation and used as innoculum for the fermentation tests.

Batch Fermentation: 4g (wet weights) of yeast inocula were transferred to 100 ml of sterile fermentation medium containing 10 or 15% molasses sugar. The inoculation rates for the 20 and 30° Brix media were 5 and 6g per 100 ml of sterile media respectively. Inoculated flasks were incubated for 3 days at 30°C, under static culture condition. The effects of supplements on the viability of the yeasts during the course of fermentation were monitored daily in supplemented media. Yeast cell viability was determined by the methylene blue method (E.B.C Analytica Microbiologiya, 1977).

RESULTS

Yeast Adaptability: Results of yeast adaptability are shown in Table 1 for the different molasses media. The rate of yeast adaptability in the unsupplemented media was as follows; 2.6±0.3d; 5.3±0.6d; 7.0±0.6d; 12.5±0.4d and 16.4±0.7d for 5, 10, 15, 25 and 30° Brix molasses media respectively. This same trend was noticed for all the supplemented media i.e. adaptability in 5°, 10°, 15°, 25° and 30° Brix molasses. Also the mean results of the effect of supplements on yeast adaptability gave the following trend; medium + soyabean (6.0d), medium + castor (6.1d), medium + groundnut (6.3d) and unsupplemented medium (8.9d).

Table I: Adaptability * of Palm Wine *Saccharomyces* Isolate J in Media of Different Molasses Sugar Concentration

Molasses Sugar Concentration (Brix)	Unsupplemented medium	Medium + soyabean	Medium + Groundnut	Medium + Castor
5	2.6±0.3	1.8±0.3	1.9±0.2	1.8±0.3
10	5.3±0.6	2.8±0.3	2.9±0.2	2.6±0.2
15	7.0±0.6	4.4±0.7	4.5±0.6	4.5±0.4
20	9.4±0.4	6.8±0.6	7.3±0.3	6.6±0.7
25	12.5±0.4	9.1±0.4	9.3±0.4	9.3±0.3
30	16.4±0.7	11.1±0.5	12.0±0.4	11.6±0.6
Mean	8.9	6.0 (33)	6.3 (29)	6.1 (31)

* Adaptability is measured as time (days) before medium effervescence was observed. Results shown are means of triplicate experiments. Figures in brackets represent % average enhancement of yeast adaptability by supplement.

Yeast Tolerance to Molasses: Results of yeast viability studies after 3 days of fermentation in the various molasses media are shown in figures 1–4 for the 10, 15, 20, and 30° Brix media respectively.

By the third day of fermentation the following viability figures were obtained for the 10° Brix media; 95% medium + soyabean; 91% medium + castor; 91% medium + groundnut; and 87% unsupplemented medium (Figure 1). At 15° Brix the yeast viability profile at the end of fermentation was as follows; 88, 94, 93 and 95% for unsupplemented, and the castor oil, groundnut and soyabean supplemented media respectively. Viability figures of 91 (groundnut and castor supplemented media), 96 (soyabean supplementation) and 88% (unsupplemented medium) were also recorded in the 20° Brix fermentation. For the 30° Brix media the unsupplemented medium recorded a viability figure of 83% while the supplemented media gave the following; groundnut 89%; soyabean 92%; and castor oil bean 87%.

Generally, a decrease in the viabilities of the yeasts was observed with increasing molasses concentration. A sharp decrease in viability was also observed within the first day of fermentation in all the media. This subsequently became more gradual over the rest of the fermentation period (figures 1–4).

Figure 5 shows the effects of molasses concentration on the viability of yeast isolate J on the third day of fermentation. It can be observed that the best yeast viability would be recorded at between 15–19° Brix molasses concentration irrespective of the nature of the supplement. Furthermore, soyabean meal supplementation conferred more tolerance to molasses than did the other supplements.

DISCUSSION

Data on yeast adaptability show that palm wine *Saccharomyces* isolate J can be successfully adapted to growth in media of up to 30° Brix unclarified molasses irrespective of the highly inhibitory nature (Lehtonen and Suomalainen, 1977) of molasses. Supplementation of adaptation medium with groundnut, soyabean or castor oil bean led respectively to a 29, 33 and 32% enhancement of yeast adaptation suggesting a possible use of these supplements as enhancers of yeast adaptation to molasses.

Viability studies results from the three-day batch fermentation indicate that *Saccharomyces* isolate J is capable of growing successfully in media of up to 30° Brix unclarified molasses and maintain good viability by the third day of fermentation. While an initial sharp fall in yeast viabilities was noticed for all the media by the 24th hour of fermentation, later falls were more gradual with medium supplementation significantly improving yeast ability to tolerate the inhibitory effects of the media. Many reports have been made on the salutary effects of soyabeans on yeast-based ethanologenesis (Damiano and Wang, 1985; Viegas *et al*, 1985; Bajpai *et al*, 1988). Results of viability studies conducted on the castor– and groundnut-supplemented media suggest that these may successfully substitute soyabean as enhancers of yeast viability during molasses-based bioethanologenic processes.

A general trend with all the fermentations, however, was the gradual improvement in yeast cell viability concurrent with rises in medium molasses sugar up to about 15–19° Brix, followed by a sharp fall which became more pronounced as the molasses sugar increased to 30° Brix. This may suggest that, as with the industrial *Saccharomyces* yeasts (Lehtonen and Suomalainen, 1977), the optimum molasses sugar concentration for palm wine *Saccharomyces* isolate J is between 15 and 19° Brix.

In conclusion, we have, in this work, established that *Saccharomyces* isolate J, a high level ethanol-tolerant palm wine isolate (Ezeogu and Emeruwa, 1993) can adapt to and ferment molasses-based media of up to 30° Brix at 30°C while maintaining good viability. We have also shown that supplementation of medium with soyabean, groundnut or castor oil bean meal (35g/l) greatly improves yeast adaptability and viability in molasses.

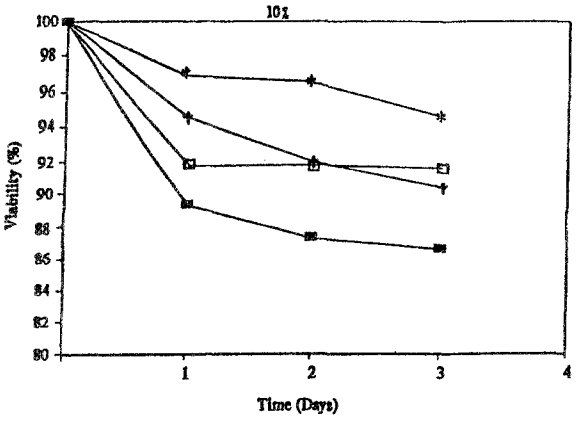


Figure 1 Effect of medium supplementation on viability of yeast isolate J in 10° Brix molasses medium over time.

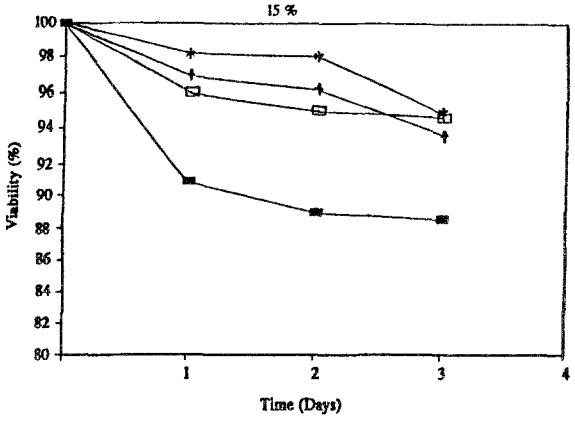


Figure 2 Effect of medium supplementation viability of yeast isolate J in 15° Brix molasses medium over time.

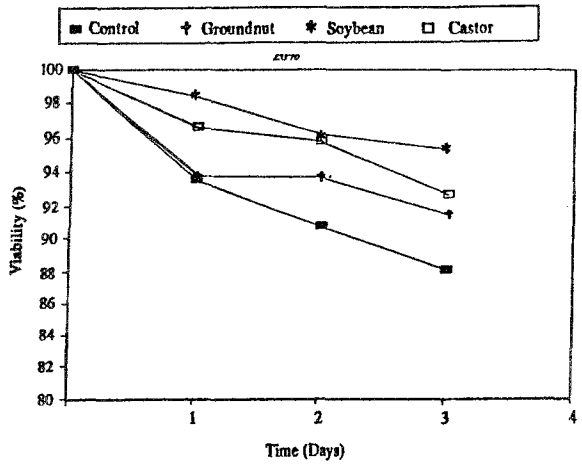


Figure 3 Effect of medium supplementation viability of yeast isolate J in 20° Brix molasses medium over time.

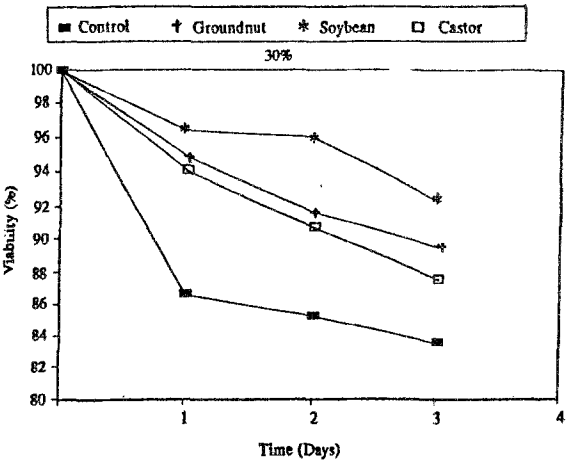


Figure 4 Effect of medium supplementation viability of yeast isolate J in 30° Brix molasses medium over time.

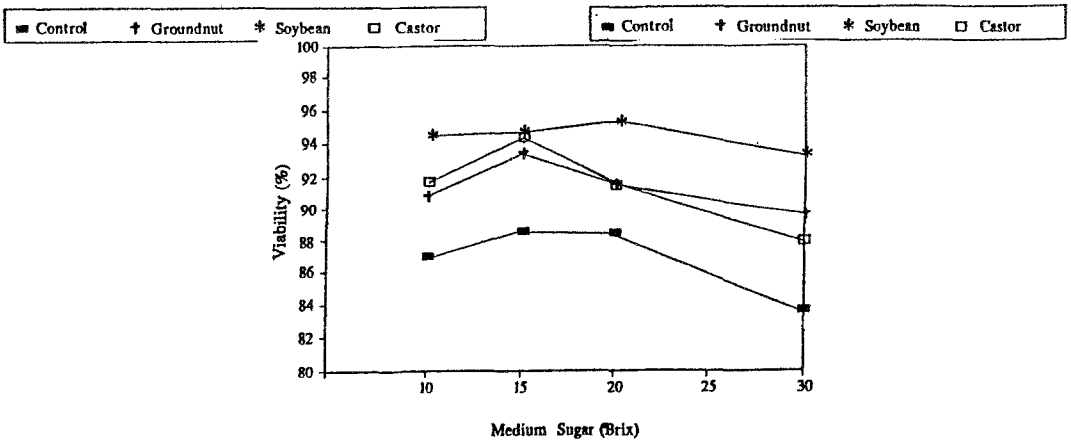
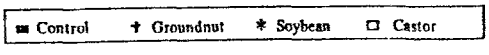


Figure 5 Effect of molasses concentration on the viability of yeast isolate J on the third day of fermentation.



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