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.

* 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; (NH₄)₂SO₄ 4g; MgSO₄.7H₂O 0.7g; KH₂PO₄ 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; MgSO₄.7H₂O 0.7g/l; KH₂PO₄ 1g/l; (NH₄)₂SO₄ 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 (2x10⁷ 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:

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\% \text{ AE} = \frac{\text{Adaptation value in unsupplemented medium}}{\text{Adaptation value in supplemented medium}} \times \frac{100}{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 inoculum 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).