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ORIGINAL ARTICLE

Production of single-cell protein from wasted date fruits by *Hanseniaspora uvarum* KKUY-0084 and *Zygosaccharomyces rouxii* KKUY-0157

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Abstract The aim of this study was to produce single-cell protein by using two yeast strains, KKUY-0084 and KKUY-0157, from spoiled date fruits. Based on the sequence of the variable D1/D2 domain of the large subunit (26S) ribosomal DNA of these strains, their identity was *Hanseniaspora uvarum* and *Zygosaccharomyces rouxii*, respectively. The two strains were assessed for their single-cell protein productivity in vitro and in a bioreactor. Both yeasts were able to utilise the juice of spoiled dates in a concentration gradient up to 25 %; however, 20 % juice was the best concentration for production of the maximum amounts of dry biomass by *H. uvarum* KKUY-0084 and *Z. rouxii* KKUY-0157 (23.5 and 20.71 g/l, respectively) at 60 h. Biomass productivity reached a maximum when the yeasts were incubated at 25 °C and pH 5.0–6.0. Addition of Mn (0.3 g/l) or Mg (0.5 g/l) had a stimulative effect on biomass production. Addition of 0.6 g/l of Mn resulted in the production of maximum dry biomass by *H. uvarum* KKUY-0084, while 0.4 g/l of the same metal was more appropriate for *Z. rouxii* KKUY-0157. Tryptone (8 g/l) as a nitrogen source increased the yield of the biomass to 34.25 and 30.75 g/l by *H. uvarum* KKUY-0084 and *Z. rouxii* KKUY-0157, respectively. In a 7-l fermentor, the highest production (48.9 g/l) of the two strains was achieved after 60 h.

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Introduction

The world deficiency in protein supply has been increasing steadily in recent years and is a main challenge facing humankind. It is important to search for new and unconventional protein sources to fill the gap between the demand and supply. Microorganisms have been used as direct and indirect sources of protein in food sources for fortification of the food supply (Wijeyaratne and Jayathilake 2000). Microorganisms have high protein content and short growth times leading to rapid biomass production, which can be continuous and is independent of the environmental conditions. The use of yeasts for single-cell protein (SCP) production is more convenient, as they can be easily propagated using cheap waste materials and easily harvested due to their bigger cell sizes and flocculation abilities (Ravindra and Anupama 2000). Yeasts are a good source of protein or amino acids. Approximately 50–52 % of the weight of dried yeast consists of protein, 30–37 % carbohydrate, 4–7 % lipids, 6–8 % nucleic acids and 7–8 % minerals (Nasseri et al. 2011).

Yeast protein is most commonly included in poultry food formulations. However, the advent of aquaculture has recently seen the emergence of ever-more sophisticated feeds for fish cultivation, and SCP of fungal origin has proved to be well digested by fish. In addition, the ingestion of yeast biomass appears to increase resistance to mycoses, which often decimate fish farms, particularly at water temperatures above 10 °C (Nell 1985).

The poultry industry has played a major role in providing animal protein (in the form of eggs and meat) to man. But the feed industry is facing immense shortages of both vegetable- and animal-based feed ingredients, which are the major

constraints in the development of this industry. For more than 10 years, broiler production has been the focus of the poultry industry, but costs of poultry feed must be reduced so that product cost may be maintained at a reasonable price. One possible option is to ferment low-cost, non-conventional agro-industrial residues to produce single-cell protein to reduce the overall production cost. The protein obtained from the micro-organisms is not only cheap but may also provide balanced nutrition. It is also a potential supplemental protein source for feeding poultry, livestock and humans (Singh et al. 1991; Pacheco et al. 1997).

Saudi Arabia is famous for having a huge number of date palm trees. It produces about 13 % of the world production of dates. There are more than six million date palm trees distributed all over the Saudi landscape, and date manufacturing is one of the most popular industries in Saudi Arabia. However, a large proportion of the dates are exposed to spoilage because of improper transporting, handling, lack of cold stores and lack of marketing. In addition, many insects are known to attack date palm fruits and trees (El-Juhany 2010). Mycotoxigenic fungi, particularly aflatoxigenic Aspergilli, have been associated with dates and date products (Shenasi et al. 2002). The spoiled or rotten dates are problematic for manufacturing, farmers and the environment. Giving the farmers a way to profit from the wasted dates may help to minimise the risk. Farmers will be happy to sell their rotten dates instead of throwing them away. The high sugar content and abundance of the wasted dates make them an important renewable agro-industrial waste material in Saudi Arabia. Their low cost is an additional factor that makes sustainable production of single-cell protein economically feasible on a large scale.

Therefore, the aim of this study was to produce single-cell protein by new yeast strains from spoiled date fruits to benefit from the agricultural wastes and reduce the total cost of single-cell protein production. Also, we optimised the cultural conditions for maximising the productivity.

Materials and methods

Yeast strains

Yeast strains (KKUY-0157 and KKUY-0084) were isolated from spoiled date samples (Berhi), collected from the Abha markets in Saudi Arabia, on yeast peptone agar medium (YPA) (Scharlau). They were identified by the sequencing of D1/D2 domain of the 26S rDNA region and phylogenetic analysis. The extraction of total yeast genomic DNA was performed according to procedures described by Hesham et al. (2006). The DNA was amplified using primers described by Kurtzman and Robnett (1998). These were NL1 (5′–GCATAT CAATAAGCGGAGGAAAAG-3′) and NL4 (5′ GGTCCGTG TTTCAAG ACGG –3′). PCR reaction was performed in a final volume of 50 μl containing GoTaq green master mix (Promega, Madison, WI, USA), 1 μl of each primer at a concentration of 0.5 mM, and 1 μl template DNA. The PCR conditions were as described by Kurtzman and Robnett (1998). The amplified DNA was purified using the GFXTM–PCR DNA and gel band purification kit (Amersham Biosciences), and the purified PCR was sequenced at Macrogen (Seoul, Korea). The DNA sequence was analysed using the DNA Blast at the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/), and the obtained nucleotide sequences was deposited in the Genbank under specific accession numbers (KC110831 and KC110836, respectively).

Date juice preparation and yeast screening

To prepare the spoilage date juice (SDJ), 200 g of stone-free date fruits were put in 500 ml of distilled water, followed by blending in a blender for 1 min at low speed, and 3 min at high speed. The homogenised extract was filtered through a double layer of cheese cloth. The residue was then washed with hot water and the solution was made up to a concentration of 20 %. Conical flasks (250 ml) containing 100 ml of SDJ were inoculated with 5 ml of desired yeast isolates (10^8 cell/ml). The inoculated flasks were incubated at 25 °C and 150 rpm for 72 h in a rotatory incubator. All treatments were performed in triplicate and arranged in a completely randomised design. The culture medium (10 ml) was transferred into a clean, weighed and dry glass tube to calculate the biomass of the yeasts as single-cell protein production. The tubes containing the culture medium were centrifuged at 10,000 rpm for 15 min. The supernatant was decanted and the pellets were dried in an oven at 80 °C for 24 h to calculate the dry weight of the biomass.

Optimisation of the conditions for single-cell protein production

Various production conditions such as incubation period (12, 24, 36, 48, 60, 72, 84 and 96 h), incubation temperature (25, 30 and 35 °C), initial pH (4, 5, 6, 7 and 8), different date juice concentrations (10, 15, 20 and 25 %), different metals (Zn, Mn, Co and Mg) and different nitrogen compounds (yeast extract, malt extract, tryptone, ammonium nitrate and ammonium dihydrogen phosphate) were optimised for *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084 in 250-ml conical flasks. Cultures were shaken at 150 rpm. Biomass production was measured at the end of each experiment as the dry weight of the cell yield.

Pilot test

The experiment was carried out in a bioreactor BioFlo/CelliGen 115 by New Brunswick, USA, with all the necessary

controls. The reactor was of 7-l capacity and the working volume was 3 l. The reactor is equipped with an agitator, pH, and temperature control systems. The reactor was cleaned and steam sterilised at 121 °C for 15 min. The sterilised medium [20 % of date juice, tryptone (8 g/l), Mg (0.6 g/l) and Mn (0.8 g/l)] containing the inoculum was transferred to the fermentor. The seed culture was grown at 25 °C for 24 h in a 250-ml flask containing 100 ml of YPD medium. The temperature of fermentation was maintained at 25±1 °C. The pH of the fermentation broth was regulated at 5.0 within pH 0.1 unit by the peristaltic pump, which injected a fine stream of sulfuric acid or sodium hydroxide. The agitator speed was maintained constant throughout the experiment at 200 rpm. The reactor was maintained under aerobic conditions. Samples were taken during the course of 96 h to monitor the dry cell weight as an indicator of single-cell protein production.

Statistical analysis

All experiments were repeated twice. With the experiment, the replicates were arranged in a completely randomised design, and the data were analysed using one-way analysis of variance (ANOVA). The significance of differences among the treatments was determined according to Least Significant Difference (LSD) ($P<0.05$) (Gomez and Gomez 1984).

Results and discussion

Biomass production potentiality from SDJ by the yeast strains

The two yeast strains, *Hanseniaspora uvarum* KKUY-8004 and *Zygosaccharomyces rouxii* KKUY-0157, were selected among 150 yeast strains isolated from different natural sources collected from the Asir region (Abha) of Saudi Arabia based on their potential to grow and propagate well on SDJ (data not shown). *H. uvarum* KKUY-8004 and 200 Z. *rouxii* KKUY-0157 emerged as the highest biomass producers from the SDJ. They produced 94.76 and 89.69 g/l of fresh biomass, and 22.18 and 20.97 g/l of dry weight, respectively (Fig. 1). Previous isolation of *Zygosaccharomyces rouxii* from sugar-rich products such as sugar syrups, honey and fruit juices (Leandro et al. 2011) and here from the date fruits proves its fructophilic affinity. It can also survive and grow in the presence of weak-acid preservatives and high concentrations of salt or sugars and tolerate high temperatures (Martorell et al. 2007) which confirms its osmotolerance characteristic. *Hanseniaspora uvarum* was frequently isolated from many natural sources (Romano 2002). In addition to its ability to ferment different sources, this yeast can generate many secondary compounds (Romano 1997). *Hanseniaspora uvarum* was detected in high cell densities during fermentation of grapes (Rojas et al. 2003). It was recorded among frutophilic species of *Hanseniaspora* yeasts (Ciani and Fatichenti 1999).

Optimisation of the production of dry biomass (SCP)

Effect of incubation period

Figure 2a shows that the highest production of dry biomass by the two yeast strains was attained after 60 h. It was noticed that the yield of *H. uvarum* KKUY-0084 was higher than that of *Z. rouxii* KKYU-0157 during the growing period. The maximum yield of *H. uvarum* KKUY-0084 was 21.35 g/l after 60 h compared with that of *Z. rouxii* KKUY-0157 (19.07 g/l). A noticeable decline in the yield of the yeast strains occurred after 72 h and slowly decreased until 96 h. These results are consistent with those obtained by Adoki (2008), who mentioned that the peak growth of *Candida* sp. on orange, plantain and banana wastes was obtained after 60 h. However, we suppose that the peak growth varies greatly depending on many factors such as yeast strain, substrate, temperature, pH, inoculum size and others.

Effect of substrate concentration

The concentration of the date syrup significantly affected the productivity of biomass of the two yeasts. The biomass yield increased proportionally with the increase in concentration of the substrate up to 20 % (Fig. 2b). The maximum yields of *H. uvarum* KKUY-0084 and *Z. rouxii* KKUY-0157 were 23.5 and 20.71 g/l, respectively, when they were grown in 20 % of date syrup after 60 h at 25 °C. When the concentration of the substrate increased up to 25 %, the yield of the two strains decreased by 50 and 41 %, respectively. These results proved that 20 % of date syrup is the most appropriate concentration to encourage the growth and production of the biomass by the two yeasts. They seemed to carry out all normal physiological processes in a moderate concentration of sugars, while the increase in date juice concentration slowed down their growth. The decrease in growth rate in high concentrations of date juice could be attributed to the viscosity of the medium and plasmolysis of yeast cells that retard or stop their growth (Pramanik 2003). In similar studies, Adoki (2008) used the optimisation of the cultural conditions for production of single-cell protein by *Candida* sp. from orange, plantain and banana wastes. He reported that the optimum substrate concentration for growth of the yeast was 2.0 % (w/v), and the growth levels were approximately equal at substrate concentrations of 1.2 and 1.5 % (w/v).

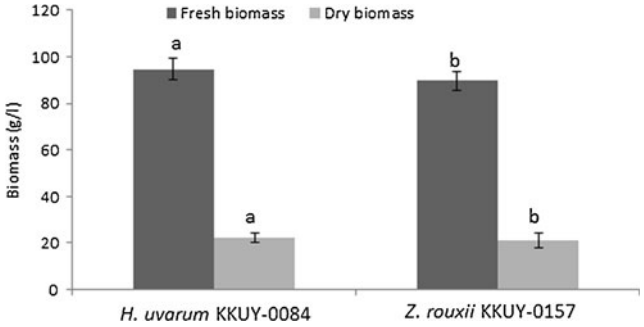

Fig. 1 Production of fresh and dry biomass from spoilage date fruits by *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084 at 25 °C after 72 h of incubation. *Columns with the same letter in the same colour* are not significant at $P<0.05$. *Bars* standard error

Effect of temperature

Figure 2c represents the effect of temperature on biomass production by *H. uvarum* KKUY-0084 and *Z. rouxii* KKUY-0157. The moderate temperature, 25 °C, was the most appropriate one for both yeasts. They were able to grow up to 35 °C; however, *Z. rouxii* KKUY-01576 was affected by the increase in temperature more than *H. uvarum* KKUY-0084. The maximum yield of *H. uvarum* KKUY-0084 was 21.96 g/l at 25 °C and decreased to 19.01 g/l at 35 °C. These results demonstrate the ability of the yeast strains to resist the increase in temperature to 35 °C; however, 25 °C is the optimum degree for biomass production. Based on the information available in the literature, the optimum temperature varies widely among the yeast strains. In this respect, Murad et al. (1992) mentioned that 28 °C is the most favourable temperature for biomass production by *Kluyveromyces lactis* grown on whey permeate. Lee et al. (1993) reported that the optimum temperature for thermotolerant *Candida tropicalis* used for SCP production was 38 °C. Rajoka et al. (2006) studied the production of SCP by *Candida utilis* at different temperatures (20–45 °C) in a stirred fermentor and reported that the maximum production of crude protein was realised when the fermentation temperature was maintained at 35 °C. They also found that the production of crude protein decreased above 35 °C. High temperature can cause inactivation of enzymes of the metabolic pathway, while low temperature may not permit the flow of nutrients across the cell membrane, resulting in a high demand for maintenance energy. However, at low temperature, the enzyme activities are expectedly low (Roels 1983; Converti and Dominguez 2001). Processing temperature is a relevant parameter affecting growth rate, oxygen diffusion and the metabolic pattern of the culture. The metabolic activity associated with substrate oxidation and biomass synthesis yields an exothermic balance, which is dependent on the type of substrate used (Forage and Righelato 1979; Cooney et al. 1969).

Effect of pH

The slightly acidic pH (4.0–6.0) was appropriate for biomass production by the two yeast strains (Fig. 2d). The yield decreased in neutral and alkaline medium. The maxima of biomass of the *H. uvarum* strain KKUY-0084 and *Z. rouxii*

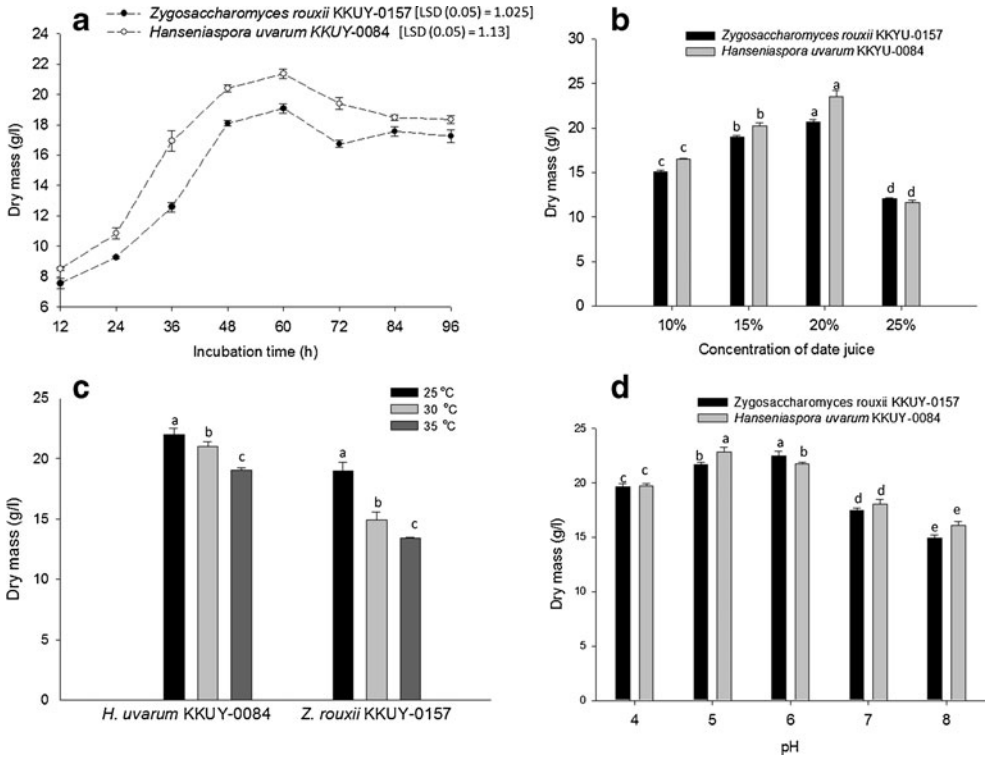

Fig. 2 Effect of different cultural conditions on single-cell production from spoilage date fruits by *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084; incubation time (**a**), concentration of date juice (**b**), temperature (**c**) and pH (**d**). *Columns with the same letter in the same colour* are not significant at $P<0.05$ (**b** and **d**). Columns with the same letter opposite to each yeast strain are not significant at $P<0.05$ (**c**). *Bars* standard error

strain KKUY-0157 were obtained at pH 5.0 and 6.0, respectively. The first strain yielded 22.8 g/l and the second isolate yielded 22.4 g/l. Our finding is supported by the suggestion that a weak acidic medium is more appropriate for the overall growth of yeasts (Pramanik 2003). The results are confirmed by those of Martorell et al. (2007), who stated that *Z. rouxii* can survive and grow in the presence of weak acid preservatives and high concentrations of salt or sugars. This unique ability is based on its high resistance to weak acids and extreme osmotolerance. Different isolates of *Z. rouxii* were able to grow at 90 % (w/v) glucose, low pH and tolerated high temperatures. Early studies of Farid (1977) showed that the maximum dry yeast weight and the protein content of *S. cerevisiae* were recorded when the initial pH was adjusted to 6.0. He noticed, that below and above pH 6.0, both dry weight and protein content decreased gradually with the decrease or increase of the initial pH values. In other cases, Abou-Zeid and Ashy (1984) and Pessoa et al. (1996) reported that the maximum production of SCP by *Candida tropicalis* on diesel oil and sugar cane bagasse hydrolyse occurred at pH 6.0. Rajoka et al. (2006) reported that pH 6.0 was the optimum for SCP production from rice polishings by *Candida utilis*. Paraskevopoulou et al. (2003) found that the optimum pH for SCP production using Kefir yeasts *Kluyveromyces, Candida, Saccharomyces* and *Pichia* was 5.5. However, other observations suggest that the pH range of any yeast strain could vary depending on the medium composition. In this context, Onishi (1963) showed that the pH range for the growth of *Z. rouxii* strain isolated from the soy sauce process without NaCl is very broad (pH 3.0–7.0), while in a medium containing 18 % NaCl, the pH range for growth is narrow (pH 4.0–5.0).

Effect of metal addition

The effect of some metals on single-cell protein production is shown in Fig. 3a. Both Mn (0.3 g/l) and Mg (0.5 g/l) significantly enhanced the growth and dry biomass of the two yeasts. The maximum yield of the *H. uvarum strain* KKUY-0084 was achieved as 25.94 and 25.22 g/l when Mn and Mg were added to the medium, respectively. Addition of either Co or Zn decreased the productivity of both strains, but it was still higher than the control yield. Addition of different concentrations of Mg or Mn induced the biomass production of the two strains. The peaks of biomass production by both yeasts were detected when 0.6 g/l of Mg was added (Fig. 3b). Addition of 0.6 g/l of Mn induced the production of biomass of *H. uvarum* KKUY-0084, while 0.4 g/l of the same metal was more appropriate for the growth of *Z. rouxii* KKUY-0157 (Fig. 3c). This reveals the importance of such metals to enhance the growth of the yeast strains but in various concentrations based on the strain requirement. Generally, various elements, such as potassium, manganese, zinc, iron and gaseous ammonia, are important to add to the medium for

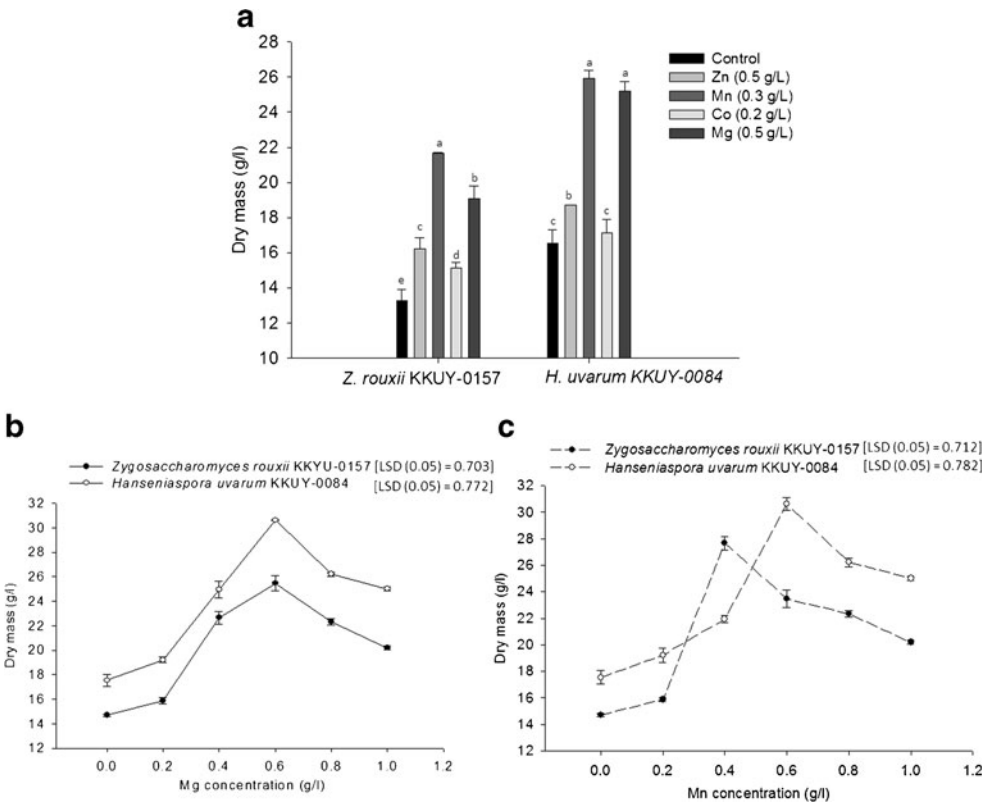

Fig. 3 Effect of metals addition on single-cell production from the spoilage date juice by *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084 (**a**), different concentrations of Mg (**b**) and different concentrations of Mn (**c**). *Columns with the same letter* opposite each yeast strain are not significant at $P<0.05$ (**a**). *Bars* standard error

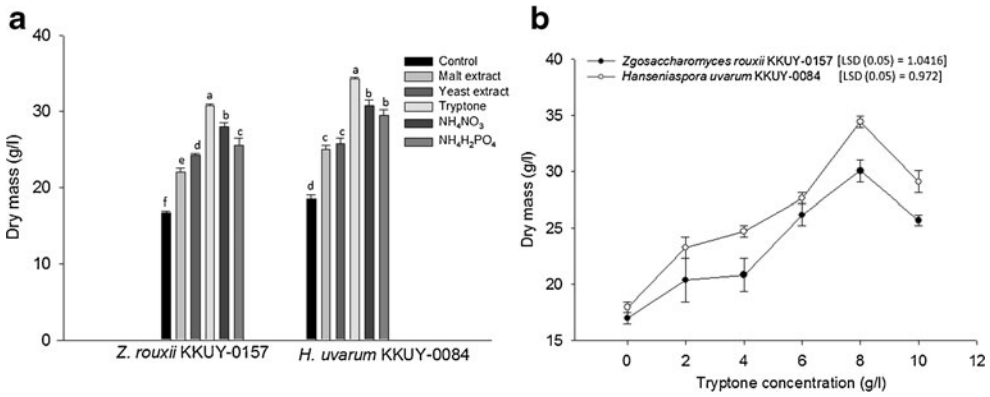

Fig. 4 Effect of nitrogen sources addition on single-cell production from spoilage date fruits by *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084 (**a**) and different concentration of tryptone (**b**). *Columns with the same letter* opposite each yeast strain are not significant at $P<0.05$ (**a**). *Bars* standard error

cultivating many microorganisms (Nasseri et al. 2011). Inorganic nutrients are assimilated through membrane transport mechanisms and are then incorporated into organic molecules (Soumalainen and Oura 1971). However, introduction of these elements in excess amount has a negative effect on the yeast cell. For example, cadmium can cause structural damage in the plasma of the membranes of the yeast cells due to binding with organic ligands (Brady and Duncan 1994).

Effect of nitrogen sources

Nitrogen sources, including three organic and two inorganic, were tested for their effects on biomass production by the two yeasts (Fig. 4a). Results showed that all nitrogen sources increased the productivity of the two yeasts significantly compared to the control. It was noticed that the organic nitrogen source increased the production of the biomass compared with the inorganic sources. Among the organic nitrogen sources, tryptone had the best result by increasing the yield of the biomass to 34.25 and 30.75 g/l by *H. uvarum* KKUY-0084 and *Z. rouxii* KKUY-0157, respectively. The optimum dose of tryptone was 8 g/l for both yeasts (Fig. 4b). Ammonia and ammonium salts are assimilable by all commonly used yeasts and fungi. Farid (1977) examined the effect of different concentrations of each of diammonium hydrogen phosphate (1.0–100.0 g/l) and yeast extract (2.0–30.0 g/l) as nitrogen sources for fodder yeast production by *S. cerevisiae*. He found that the maximum levels of dry biomass were recorded at 0.5 % $(NH_4)_2HPO_4$ or 1.2 % yeast extract, while the highest total protein concentrations were obtained at 0.4 % $(NH_4)_2HPO_4$ or 1.0 % yeast extract. Moeini et al. (2004) studied the improvement of SCP production from whey using mixed yeast cultures and tested the effect of the addition of 0.9 g/l of ammonium sulphate as nitrogen supplementation to the growth medium; this addition had a significant effect on increasing SCP production.

Pilot test

The productivity of both strains was assessed in a 7-l fermentor with optimisation of all conditions. The results showed that the maximum production was achieved at 60 h for both strains to corroborate the results obtained from conical flask experiments. Both strains gave the highest yield (48.9 g/l) after 60 h of incubation (Fig. 5). The production gradually decreased after this time. The results indicate that biomass production in batch culture is significantly higher compared to the production in conical flasks. This could be due to the large quantity of substrate and availability of other factors such as dissolved oxygen, which induces the vegetative growth of yeast.

Based on our results, we conclude that *Hanseniaspora uvarum* KKUY-0084 and *Zygosaccharomyces rouxii* KKUY-0157 are good producers of single-cell protein from spoiled date fruits. They showed the best growth and production of biomass at 25 °C in a 20 % date juice concentration, they could resist an increase in temperature to 30 °C, and they could grow in higher concentrations of date juice. We noticed that the growth and biomass productivity of the two strains were greatly enhanced by adding metals such as Mn or Mg as well as a nitrogen source (tryptone). We encourage researchers to seek new yeasts that have the ability to produce large

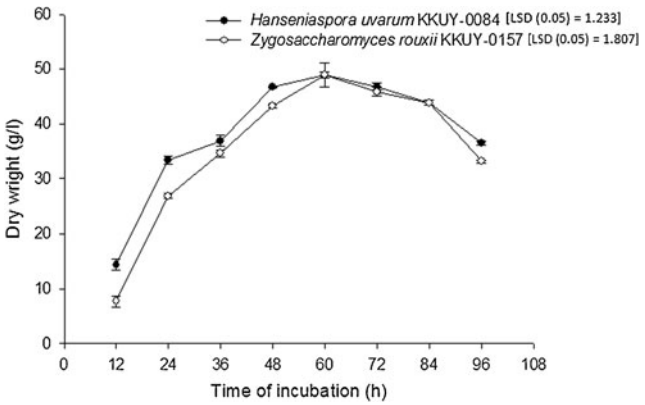

Fig. 5 Single-cell protein production in batch culture from spoilage date fruits by from spoilage date fruits by *Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084. *Bars* standard error

amounts of single-cell protein to narrow the gap between demand and production. Based on our results, we propose that it is very important to study the cultural and nutritional conditions for each new yeast to maximise productivity.

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References

Abou-Zeid AZA, Ashy MA (1984) The potential of yeast for the production of single cell protein. Arab-Gulf J Sci Res 2(1):315–326

Adoki A (2008) Factors affecting yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp. Afr J Biotechnol 7(3):290–295

Brady D, Duncan JR (1994) Cation loss during accumulation of heavy metal by *Saccharomyces cerevisiae*. Biotechnol Let 16:543–548

Ciani M, Fatichenti F (1999) Selective sugar consumption by apiculate yeasts. Lett Appl Microbiol 28:203–206

Converti A, Dominguez JM (2001) Influence of temperature and pH on xylitol production from xylose by *Debarryomyces hansenii*. Biotechnol Dioeng 75:39–45

Cooney CL, Wang DIC, Mateles RI (1969) Measurement of heat evolution and correlation with oxygen consumption during microbial growth. Biotechnol Bioeng 11:269–281

El-Juhany L (2010) Degradation of date palm trees and date production in Arab countries: causes and potential rehabilitation. Aust J Basic Appl Sci 4(8):3998–4010

Farid MA-F M (1977) Chemical and biochemichal studies on the by-products remaining after onion dehydrating industry. MSc Thesis, National Research Centre, Riyadh

Forage AJ, Righelato RC (1979) Biomass from carbohydrates. In: Rose AH (ed) Microbial biomass. Economic microbiology, vol 4. Academic Press, London, p 289

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. Wiley, New York, ISBN: 13-9780471879312

Hesham A, Wang Z, Zhang Y, Zhang J, Lv W, Yang M (2006) Isolation and identification of a yeast strain capable of degrading four and five ring aromatic hydrocarbons. Ann Microbiol 56:109–112

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73:331–371

Leandro MJ, Sychrová H, Prista C, Loureiro-Dias MC (2011) The osmotolerant fructophilic yeast *Zygosaccharomyces rouxii* employs two plasma membrane fructose uptake systems belonging to a new family of yeast sugar transporters. Microbiology 157:601–608

Lee C, Yamakawa T, Kodama T (1993) Rapid growth of thermotolerant yeast on palm oil. Biotechnology 9:187–190

Martorell P, Stratford M, Steels H, Fernández-Espinar MT, Querol A (2007) Physiological characterization of spoilage strains of *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* isolated from high sugar environments. Int J Food Microbiol 114:234–242

Moeini H, Nahvi I, Tavassoli M (2004) Improvement of SCP production and BOD removal of whey with mixed yeast culture. Electric J Biotechnol 7:249–255

Murad HA, Abd-El-Ghani S, El-Shenawy K (1992) Bioconversion of whey permeates into *Kluyveromyces lactis* biomass. Egyptian J Dairy Sci 20:261–271

Nasseri AT, Rasoul-Amini S, Moromvat MH, Ghasemi Y (2011) Single cell protein: production and process. Am J Food Technol 6:103–116

Nell JA (1985) Comparison of some single cell proteins in the diet of the Sidney rock oyster (*Saccostrea commercialis*). Prog Fish Cult 47: 110

Onishi H (1963) Osmophilic yeasts. Adv Food Res 12:53–94

Pacheco MT, Caballero-Cordoba GM, Sgarbieri VC (1997) Composition and nutritive value of yeast biomass and yeast protein concentrates. J Nutr Sci Vitaminol 43:610–612

Paraskevopoulou A, Athanasiadis I, Kanellaki M, Bekatorou A, Bletas G, Kiosseoglou V (2003) Functional properties of single cell protein produced by Kefir microflora. Food Res Int 36:431–438

Pessoa AT, Mancilha IM, Sato S (1996) Cultivation of candida tropicalis on sugar cane hemicellulose hydrolyzed for microbial protein production. J Biotechnol 51:83–88

Pramanik K (2003) Parametric studies on batch alcohol fermentation using *Saccharomyces* yeast extracted from toddy. J Chin Inst Chem Engrs 34:487–492

Rajoka MI, Khan SH, Jabbar MA, Awan MS, Hashim AS (2006) Kinetics of batch single cell protein production from rice polishings in continuously aerated tank reactors. Bioresour Technol 97:1934–1941

Ravindra P, Anupama (2000) Value-added food: single cell protein. Biotechnol Adv 18:459–463

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier, Amsterdam

Rojas V, Gil J, Piñaga F, Manzanares P (2003) Acetate ester formation in wine by mixed cultures in laboratory fermentations. Int J Food Microbiol 86:181–188

Romano P (1997) Metabolic characteristics of wine strains during spontaneous and inoculated fermentation. Food Technol Biotechnol 35: 255–260

Romano P (2002) Role of apiculate yeasts on organoleptic characteristics of wine. In: Ciani M (ed) Biodiversity and biotechnology. Research Signpost, Trivandrum

Shenasi M, Aidoo KE, Candlish AAG (2002) Microflora of date fruits and production of aflatoxins at various stages of maturation. Int J Food Microbiol 79:113–119

Singh A, Abidi AB, Agrawal AK, Darmwal NS (1991) Single cell protein production by *Aspergillus niger* and its evaluation. Zentralbl Mikrobiol 146:181–184

Soumalainen H, Oura E (1971) Yeast nutrition and solute uptake. In: Rose AH, Harrison JS (eds) The yeasts, vol 2, 1st edn. Academic, London, p 3

Wijeyaratne SC, Jayathilake AN (2000) Characteristics of two yeast strains (*Candida tropicalis*) isolated from Caryota urens (Kithul) toddy for single cell protein production. J Natl Sci Found Sri Lanka 28:79–86