American Journal of Food Science and Health
Vol. 3, No. 4, 2017, pp. 75-82
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Screening of Thermotolerant Yeasts in the Sudan

Iman Mohammed Ali[1], Sara El Kheir Mustafa[2, *], Fatin Hassan Farahat[1], Abdel Mutalab Mohammed Khater[1]

[1]Food Science and Rural Development Department, Faculty of Agriculture, Omdurman Islamic University, Omdurman, Sudan
[2]Nutrition and Food Technology Department, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan

Abstract

A total of Seventy four (74) thermotolerant yeast strains were isolated from 67 food samples taken from different areas in the Sudan. Among the collected yeasts 47 isolates were regarded as thermophilic, 5 isolates regards as extremely thermopilic because they grow at 20°C and 49°C. The yeasts described as extremely thermophilic were identified to the genus and species level according to Lodder and Barnett. Three isolates were assigned to species *Saccharomyces cereivisae* and two isolates were assigned as *Kluyveromyces ssp*. The optimum growth temperature ranged between 30°C and 40°C. The extremely thermophilic yeast isolates were used in fermentation tests for the production of ethanol. The isolate identified as *Kluyveromces sp* gave final ethanol concentrations in the mash of 6% (w/v). This amount represented a yield of 83% of the theory. The other isolates gave ethanol concentrations of 3% to 2.5% (w/v) representing yields of only 50% of the theory. This indicates that these isolates area not suitable for ethanol production. Being thermotolerant they could be of interest for single cell protein production.

Keywords

Thermotoleant Yeast, Thermophilic Yeast, *Saccharomyces cereivisae*

Received: May 12, 2017 / Accepted: July 4, 2017 / Published online: August 1, 2017

1. Introduction

Sudan is a hot country with day temperatures in summer going far above 40˚Cin such a hot climate, thermotolerant and thermophilic microorganisms are likely to dominate. Studies done so far support this assumption [1; 2]. Thermotolerant and thermophilic microorganisms are associated with processing of fermented foods, with food spoilage and with diseases of humans, animals and plants. Therefore, they are of great economic importance and hence it is highly important that these microorganisms should be screened for and identified so that proper propagation methods for the useful ones could be developed and suitable control methods for the harmful ones could also be established. Moreover, thermophilic and thermotolerant microorganisms are important in the industry. The use of such microbes in the industry will lead to reduction in the cooling cost. These microbes could be used directly in the fermentation industry or could be used as a source of genus that confer this property on other microbes used in thefermentation industry. Therefore, it is important to screen for such microbes and to seek for their thermotolerance genes and prepare gene banks from them. Yeasts are widely used in the industry especially for the production of baker's yeast single cell protein and ethanol. Such industries convert cheap raw materials such as molasses, whey, sulphiteliquor etc. to products of economic importance for example foods, feeds and fuels. Most of the yeast strains used in these industries are mesophilic, with optimum growth temperatures of about 30°C such yests suit the cold climates of European and American countries. In hot climates such as those prevailing in Africa in general and in the Sudan in particular, the use of

* Corresponding author
E-mail address: sara.elkheir@gmail.com (S. El K. Mustafa)

such mesophilic yeasts in the fermentation industry necessitates the application of intensive cooling. This will add much to the cost of production andwill put a heavy burden on the meager energy resources of the country. The end effect will be a retardation in the establishment of these industries in the Sudan. The use of Thermotolerant and thermophilic yeasts in these industries will remove this constraint and the fermentation industry can find a good start in this country. Theromotolerant and thermophilic yeasts are also important in food spoilage [3; 4 and 2] isolated many types of thermotolerant and themophilic yeasts from spoiled food that was subjected to processing. This indicates that yeasts weren't killed by the pasteurization processes employed in these factories. Some of these thermotolerant yeasts, isolated from spoiled processed foods were potential pathogens [2]. Therefore, it is very important to study the presence of these yeasts in foods so as to have an idea about the extent of food contamination with these microbes and to be able to develop proper pasteurization methods that eliminate them from our foods. This study aims to Screen thermotolerant and thermophilic yeasts in different habitats in the Sudan, with special emphasis on foods, identification of the most thermotolerant yeasts among the collection and determination of the optimum, minimum and maximum growth temperatures of these yeasts.

2. Material and Methods

2.1. Collection of Samples

Samples were collected from food processing factories, households markets and flowers. Liquid samples were taken in to sterile Duran bottles with screw caps and solid samples in sterile polythene bags. Samples taken from areas near Khartoum were transported on the same day to the faculty of agriculture in Khartoum north and the yeasts were isolated immediately. Samples from Kenana were transported to the faculty of agriculture and the isolation of the yeasts was carried out on the same day of arrival.

2.2. Isolation of Yeasts from Samples

The yeasts were isolated by the direct plating of the samples or their suspensions on a medium of malt and yeast extract agar according to[2].

2.3. Purification of the Yeast Isolates

Different yeast colonies were purified by inoculating them onto solidified yeast extract and malt extract agar plates in quadrantic streaks manner, then they were incubated at 40°C for 48 hours. The streaking was repeated un till pure yeasts cultures were obtained. Pure yeasts cultures were transferred ontoslants of the same medium and incubated at 40°Cfor 48 hours. After that the slants were kept in a refrigerator for further studies. Sub culturing was performed periodically every 1- 2 months.

2.4. Screening for Thermotolerant Yeasts

The yeast isolates were grown on plates of the malt extract-yeast extract agar medium at incubation temperatures of 42°C, 46°C, 49°C and 50°C. The yeasts that showed growth at these temperatures were selected for further studies.

2.5. Determination of the Optimum, Minimum and Maximum Growth Temperatures

The yeasts isolates that proved to be thermotolerant as a result of the previous tests were subjected to tests for the determination of their optimum, minimum and maximum growth temperatures. The yeasts were grown in 500 ml. Erlenmeyer flasks containing 300ml. Malt extract- yeast extract broth. The media were inoculated with quantities of inocula that gave the same amount of initial absorbance. The flasks were incubated at temperatures of 19°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 49°C and 50°C.

The growth was followed by measuring the absorbance in a spectrophotometer (Dr/ 3 spectrophotometer model 41700/41800) at a wave length of 640 nm [5].

2.6. Identification of Yeasts

Selected yeast isolates were identified according to the methods described by [5;6; 7; 8 and 9].

2.7. Ethanol Production

The medium used was a urea molasses medium (1% sodium dihydrogen phosphate+ 0.35% urea+ molasses with 14.5 w/v sugar concentration, modified from [10]. The pH was 5.02 the fermentation was done in flasks each containing 400ml of medium. The flasks were incubated at room temperature and were shaken before sample taking. Samples were taking every hours and the ethanol concentration was determined by two methods: according to [11]andweighing method

2.8. Sterilization of Media and Glassware

The malt and yeast extract and the molasses media were sterilized in the autoclave at 121°C for 15min. The sugar solutions were sterilized by tandalization and ethanol by filtration. Glassware were sterilized in the oven at 160°C for 3 hours. Paraffin – wax layer was sterilized at 160°C for 2 hours.

2.9. Yeast Concentration

Yeast concentration was determined by using a spectrophotometer (DR/3 Spectrophotometer model 41700/41800) at a wave length of 460nm according to [5].

2.10. Alcohol Determination by Caputi and Wright Chemical Method

According to [11] one ml of sample was steam- distilled into acidified K_2 Cr_2O_7 solution of known volume and concentration. Oxidation of ethanol (EtOH) to formic acid (HOAC) was completed by heating. Unreacted dichromate was determined by titration with standers Fe $(NH4)_2$ $(SO4)_2$ solution using phenolphthalein as indictor. Kjeldahl apparatus was used.

3. Result and Discussion

Seventyfour pure yeast cultures were obtained at the end of the screening programme as shown in table 1. All of these yeast isolates were grown at 40°C and hence they can be regarded as themotolerant yeasts [12]. It is interesting to notice that almost all samples collected contained thermotolerant yeasts with some samples containing more than one yeast type these shows how thermotolerant yeasts are abundant in the Sudanese's environment. Because most of these yeasts were isolated from food materials, this condition makes clear that yeasts contribute much to food spoilage and to food fermentation.

Table 1. General view of the yeast isolates.

Source of samples	Number of samples	No. of pure yeast cultures
Market	10	11
Sugar factory	21	19
Dairy plant	15	22
Food processing factory	11	10
Households	09	11
Flower	01	01

3.1. Ability of the Isolated Yeasts to Grow at Elevated Temperatures

The ability of the isolated yeasts to grow at elevated temperatures was tested by growing the yeast isolates in petri dishes containing malt extract and yeast extract agar at temperatures starting with 40°C. The results are shown in Table 2. As can be seen from this table, all isolates showed growth at 40°C and42°C only three out of the 74 isolates tested didn't show growth and at 46°C only 25 isolates didn't show growth. There were only 5 isolates which showed growth at 49°C and there was no growth at 50°C at all. According to [12] mesophilic yeasts have a temperature growth range between 5°C and 35°Cthermotolerant yeast between 8°C and 42°C and thermophilic yeasts between 20°C and 46°C. Therefore, all of the 74 isolates can be regarded as thermotolerant and 47 isolates were thermophilic. The 5 isolates that showed growth at 49°C are extremely thermophilic yeasts. Such yeasts are highly interesting for the biotechnologist for the food microbiologist and for the genetic engineer. They can be used in the fermentation industry, have potentially dangerous food spoilers and they are an attractive source of genes. [2] tested 200 yeast isolates collected from different areas in the Sudan. Eighty of these isolates were classified as thermotolerant; because they were not killed by heating at 55°C for 20 minutes and 46 out of these 80 isolates were classified as thermophilic; because they grew at 45°C and 50°C.

Table 2. Yeast isolates and their ability to grow at different elevated temperatures.

Sample source	40°C	42°C	46°C	49°C	50°C
Saccharumofficinrum	+	+	-	-	-
Milk (spoiled)	+	+	-	-	-
Yogurt	+	+	+	-	-
P. guava L	+	+	-	-	
Hulu Mur	+	+	-	-	-
Milk (spoiled)	+	+	+	-	-
Marisa	+	+	-	-	-
P. guava L	+	+	-	-	-
Hameliaspherocarpa	+	+	-	-	-
M. indica L	+	+	-	-	-
M. sapientum L	+	+	-	-	-
Sharbout	+	+	-	-	-
Pickled (carrot/cucumber)	+	+	+	-	-
M. indica L	+	+	-	-	-
Citrus paradise L	+	+	-	-	-
P. dactylifera L	+	+	-	-	-
Hibiscus sabdariffa1 L	+	+	+	-	-
Hibiscus sabdariffa2 L	+	+	+	-	-
P. dactylifera1 L	+	+	+	-	-
P. dactylifera2 L	+	+	-	-	-
P. dactylifera3 L	+	+	+	-	-
Cucurbitamoschata L	+	+	+	+	-
Jam concentrate	+	+	+	+	-
Canned jam 1	+	+	+	+	-
Canned jam 2	+	+	+	-	-
Mango juice	+	-		-	-
Ketchup	+	+	+	-	-
Souse raw material 1	+	+	+	-	-
Souse raw material 2	+	+	-	-	-
Souse concentrate 1	+	+	+	-	-
Souse concentrate 2	+	+	-	-	-
Butane milk	+	+	+	+	-
Milk (pasteurized 1)	+	+	-	-	-
Milk (pasteurized 2)	+	+	+	-	-
Milk (pasteurized 3)	+	+	+	-	-
Butane yogurt	+	+	+	+	-

Table 2. Continued.

Sample source	40°C	42°C	46°C	49°C	50°C
Butane mish 1	+	+	-	-	-
Butane mish 2	+	+	+	-	-
Yogurt	+	+	+	-	-
Butane (milk + yogurt)1	+	+	+	-	-
Butane (milk + yogurt)2	+	+	+	-	-
Butane washing water	+	+	+	-	-
Yogurt before incubation	+	+	-		-
Butane milk cheese1	+	+	+	-	-
Butane milk cheese2	+	+	+	-	-
Kuku milk	+	-	-	-	-
Cheese end product 1	+	-	-	-	-
Cheese end product 2	+	-	-	-	-
Kuku washing water	+	+	+	-	-

Sample source	40°C	42°C	46°C	49°C	50°C
Kuku butter + yogurt	+	+	+	-	-
Kuku butter 1	+	-		-	-
Kuku butter 2	+	+	+	-	-
Kuku butter 3	+	+	+	-	-
Sugar cane (burned)	+	+	+	-	-
Crusher diluted	+	+	+	-	-
Impipition water 1	+	+	-	-	-
Impipition water 2	+	+	-	-	-
Sewage water	+	+	-	-	-
Bagasse 1	+	+	+	-	-
Bagasse 2	+	+	+	-	-
Sugar juice 1st mil 1	+	+	+	-	-
Sugar juice 1st mil 2	+	+	+	-	-
First crusher from 1st mill	+	+	+	-	-
Gelatinize sugar	+	+	+	-	-
Extract from 1st mill	+	+	+	-	-
Molasses (fermented)1	+	+	+	-	-
Molasses (fermented)2	+	+	+	-	-
Mud (certified juice)1	+	+	+	-	-
Mud (certified juice)2	+	+	-	-	-
Extract from 1st mill 1	+	+	+	-	-
Extract from 1st mill 2	+	+	-	-	-
Extract from 1st mill 3	+	+	-	-	-
Kisra	+	+	-	-	-

These thermophilic yeasts were isolated from different areas in the Sudan. They included species like *Kluyveromyces marxianus, Candida Kefyer* and *Hansenula polymorpha*. If these yeasts are used in the fermentation industry, the advantages described by [13] will be realized. These advantages include the decreased costs for cooling equipment, reduction of energy costs needed for cooling, easier handling in regions with warmer climates and fewer problems with yeast contamination. [10; 2] used thermotolerant and thermophilic yeasts for the production of ethanol and SCP. From laboratory experiments and from calculations with simulated data, [2] found results that support the benefits mentioned by [13] in using thermophilic yeasts in the industry. Thermotolerant and thermophilic yeasts are also dangerous potential food spoilers, because they can stand mild pasteurization temperatures. [3; 2] isolated thermophilic and thermotolerant yeasts from many foods, such as spoiled milk and milk products and different beverages. The most dominant thermophilic and thermotolerant yeast isolates by these authors were *Issatchenkiaorientalis, Candida krusel, Kluyveromyces marxianus, Candida kefyer, Hansenula polymorpha, Debaromyces hansenii, Candida famata, Pichia membra enefaciens, Candida valida* and *Saccharomyces ssp*. Some of these thermophilic and thermotolerant yeasts are also potential pathogens such as *L. orientalis, C. krusel, Debaromyces hansenii* and *C. Famata.* This constitutes an additional health hazard to the public. These yeasts are also an important source of genes that code for these characters. Gene banks could be made and stored for different purposes.

3.2. Determination of the Growth Temperature Range

The extremely thermophilic yeast isolates were grown at the temperatures 20°C, 25°C, 30°C, 35°C, 40°C, 46°C, and 49°C to determine their optimum, maximum and minimum temperatures of growth as described in the methods. The results are presented in figures 1, 2, 3, 4, and 5. The minimum and maximum growth temperatures for the 5 extremely thermophilic yeast isolates with the codes A, B, C, D and E are 20°C and 49°C respectively. The minimum growth temperature of these yeasts is similar to that suggested by [12] for thermophilic yeasts, but their maximum growth temperatures are higher than the 46°C suggested by them. For this reason, these yeasts can be regarded as extremely thermophilic. The optimum temperature for the growth of isolates A and B seems to be 30°C, because they gave the highest amount of growth at this temperature. The second best amount of growth was given at 35°C followed by 40°C and then 25°C and 46°C (figure 1 and 2). On the other hand, isolates C, D and E seem to have 40°C as their optimum temperature for growth. The second best temperature for this group was 35°C followed by 30°C and then 46°C and 25°C (figure 3, 4 and 5).

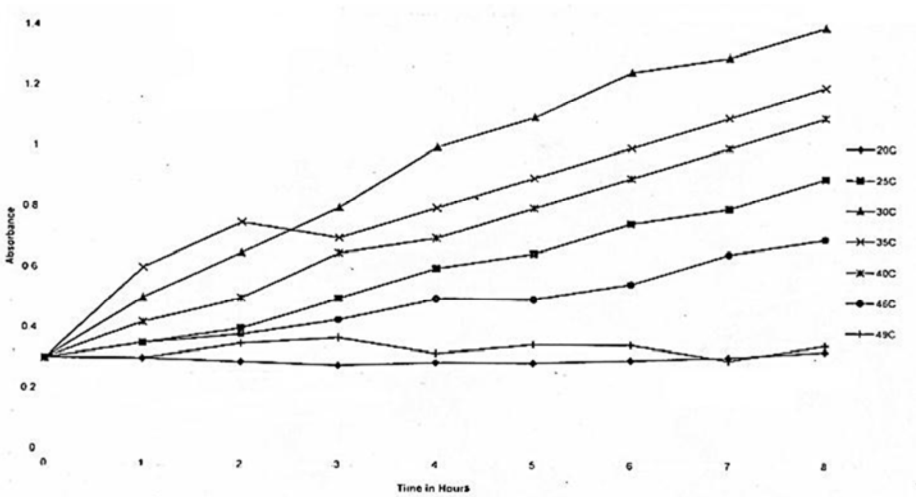

Figure 1. Growth Curves of Sample: "A" at Different Temperature.

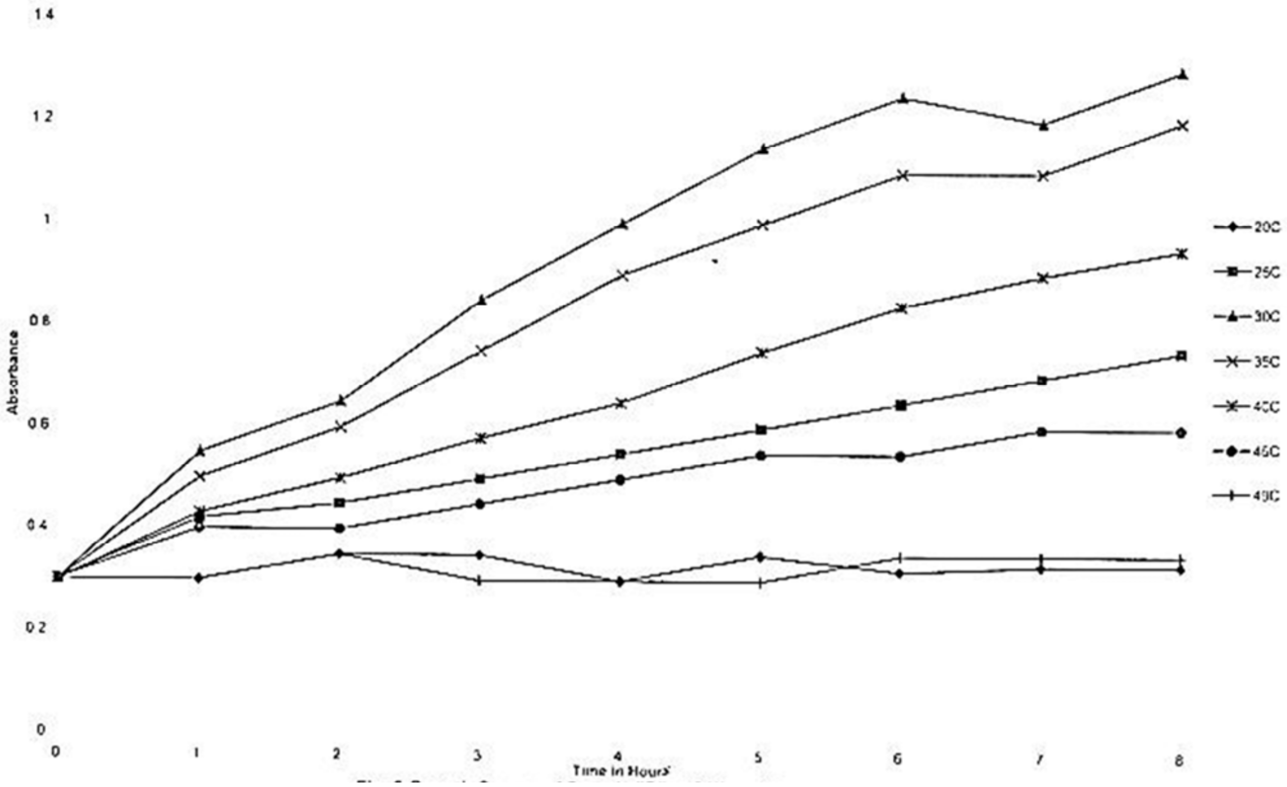

Figure 2. Growth Curves of Sample "B" at Different Temperatures.

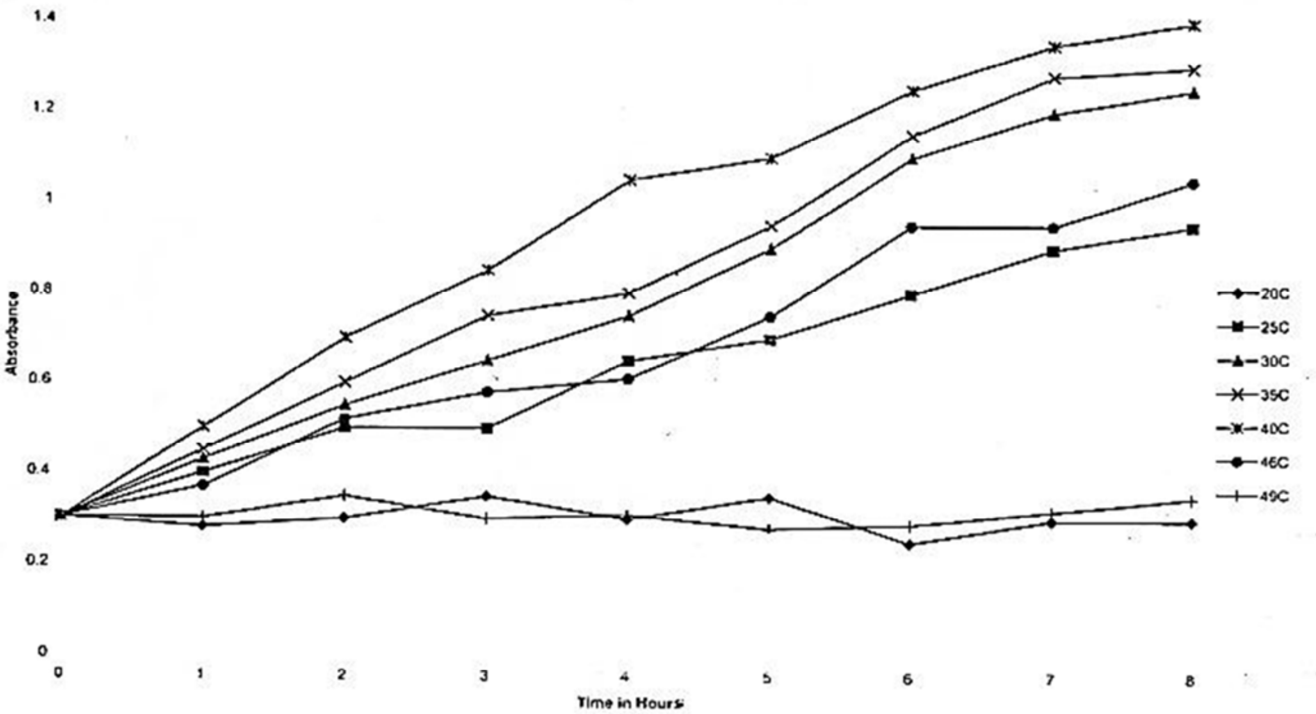

Figure 3. Growth Curves of Sample "C" at Different Temperatures.

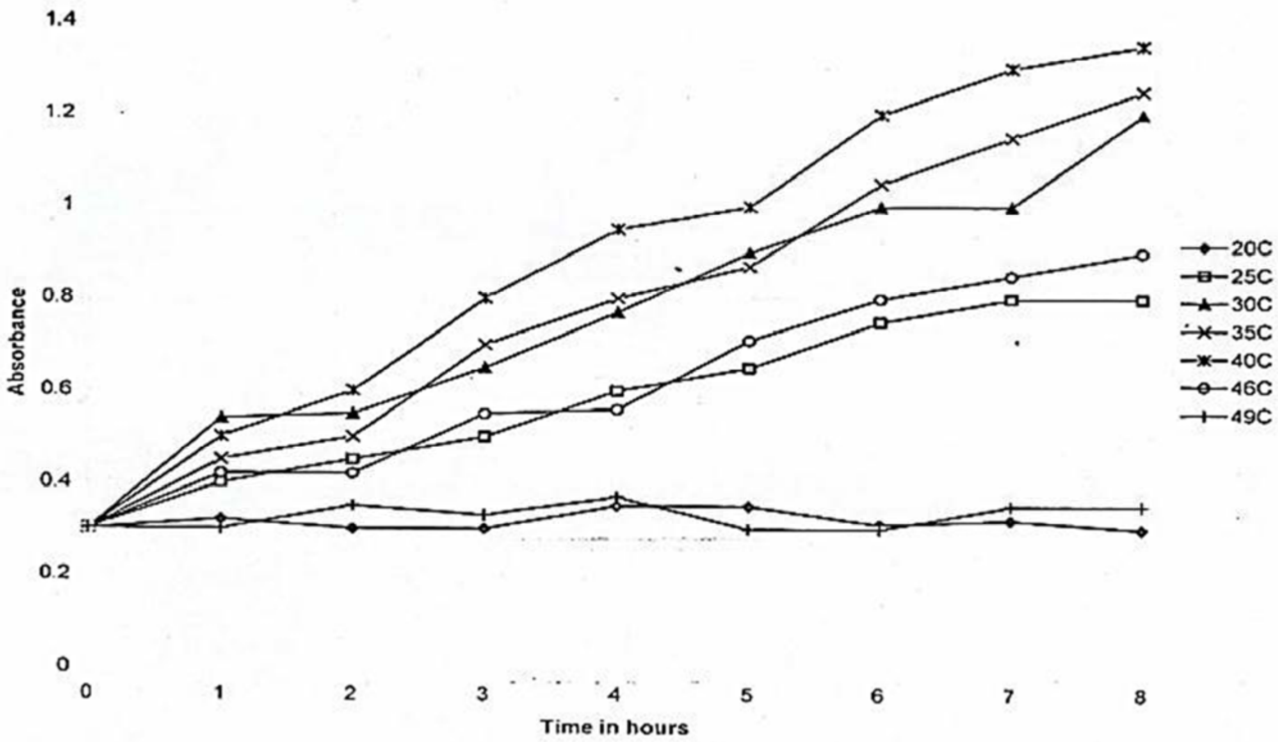

Figure 4. Growth Curves of Samples "D" at Different Temperatures.

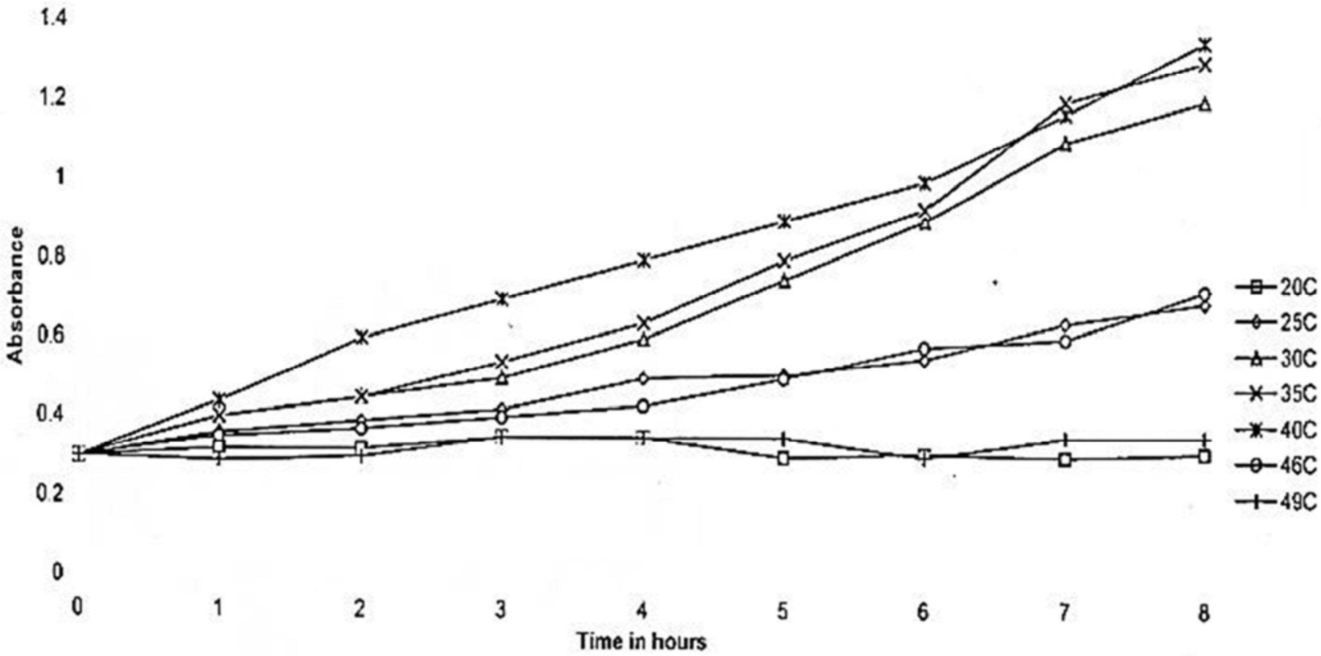

Figure 5. Growth Curves of Sample "E" at Different Temperatures.

It is worth mentioning that these yeasts grew better at 46°C than at 25°C this is another indication of their thermophilicity. These findings are in agreement with results obtained by [2] for the extremely thermophilic yeasts C. kefyer and Kluyveromyces marxiamus. The other extremely thermophilic yeast described by [2] namely *Hansenula polymorpha* had 40°C as its optimum growth temperature, but it gave better growth at 45°C that at 35°C, and also it grew at 50°C.

3.3. Yeast Identification

Four yeast isolates were subjected to preliminary identification tests that included morphological and physiological characters. The results are presented in table 3. Using identification keys in [6;5 and 14]the isolates were tentatively identified as:

A: *Saccharomyces cerevisiae*

B*: Saccharomyces cerevisiae*

C: *Saccharomyces cerevisiae*

D: *Kluyveromyces ssp*.

E:*Kluyveromyces ssp*.

As can be seen in table 3 these yeasts can grow aerobically and un aerobically on many sugars as carbon and energy sources. They are also osmotolerant, and in addition, they are thermophilic. These properties make them dangerous potential food spoilers. These same characteristics also make these yeasts of potential technological importance, for example for the production of SCP, ethanol of baker's yeast. [3]reported on the presence of yeasts in carbonated beverages in the Sudan. They found among these spoilers some thermophilics that grew at 44°C. [2] isolated thermophilic yeasts from spoiled milk and from the line of production in different food factories that process fruits and vegetables and from the line of production in a sugar factory. The extremely thermophilic yeast *Hansenula polymorpha*that grew at 50°C was isolated from different stages of the line of production in a sugar factory, especially in a juice of 60% sugar concentration. Physiological tests proved the osmotolerance of this yeast and in addition it was found that this yeast could assimilate under aerobic conditions, many hexoses and pentoses. It can also assimilate the organic acids succinate and citrate, which are widely used as preservatives in the food industry. This yeast is therefore, a dangerous food spoiler. Another yeast described by [2] was *Kluyveromyces marxianus* and it's a sexual state *Candida Kefyer*. This yeast was isolated from a spoiled milk and from the line of production in many food factories. It was also extremely thermophilic (grew at 47°C) and osmotolerant (grew on 50% glucose). It was able to assimilate aerobically and an aerobically many hexoses, pentoses and polysaccharides such as lactose and inulin. In addition to the organic acids lactate, succinate and citrate. It is, therefore, also an extremely dangerous food spoiler. These findings support the results obtained in our work. It is obvious that many of the existing yeast species are food spoilers causing food losses of unknown dimensions.

Table 3. Some morphological and physiological characters of the yeast isolates.

Colony	A	B	C	D	E
Shape	Circular	Circular	Circular	Circular	Circular
Color	White	White	White	White	White
Surface	Smooth	Smooth	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Convex	Convex
Cells	A	B	C	D	E
Shape	Oval	Oval	Oval	Oval	Oval
Division	Budding	Budding	Budding	Budding	Budding
Hypha	Pseudohyphae	Pseudohyphae	Pseudohyphae	Pseudohyphae	Pseudohyphae
Spores	A	B	C	D	E
Shape	Spherical	Spherical	Spherical	Oval	Oval
Number	1-2	1-2	1-2	1-2	1-2
Fermentation	A	B	C	D	E
Glucose	+	+	+	+	+
Sucrose	+	+	+	+	+
Raffinose	-	+	+	+	+
Maltose	-	-	-	+	+
Lactose	-	-	-	-	-
Galactose	+	+	+	+	+
D-Ribose	-	-	-	-	-

Table 3. Continued.

Growth	A	B	C	D	E
Sucrose	+	+	+	+	+
Maltose	-	-	-	+	+
Raffinose	+	+	+	+	+
D-Galactose	+	+	+	+	+
Ribose	-	-	-	-	-
Lactose	-	-	-	-	-
Ethanol	+	+	+	+	+
Methanol	+	+	+	+	+
Nitrate	-	-	-	-	-
Starch formation	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-
33.3% Glucose	+	+	+	+	+
37.5% Glucose	+	+	+	+	+

3.4. Ethanol from Molasses

Four of the yeast isolates namely three strains of *Saccharomyces cerevisiae* and *Kluyveromyces sp* were used for ethanol production as described in the methods and the results are represented in Fig. 6.

The strain *Kluyveromyces sp* gave 7.6% (v/v) ethanol in unshaken flask fermentation, representing a yield of 83% of the theoretical one. This yield is quite reasonable because the fermentation conditions were not optimum. Shake flask condition are better and a stirred bio-reactor is the optimum condition. In addition, yields in fed batch fermentation are higher than yields in batch fermentation. [2] found that the yield increased from 92% in batch fermentation to 97.7% in fed batch fermentation. [15] described yeasts that gave yields of 94% to 69% and find alcohol concentration in the fermentation broth of 6.8% to about 4% (w/v) the best yeasts described by [2] gave under optimum fermentation conditions yields of 97.7% and final alcohol fermentation in the fermentation broth of 7.9% (w/v). [16]reported about commercial yeast strains that gave ethanol concentration in the broth between 7.5% (w/v) and 7.7% (w/v) representing yields of 98% to 90% of the theory. [10] used yeasts isolated from different habitats in the Sudan for ethanol production in flask experiments. The highest ethanol concentration recorded in these experiments was 5.24% without indicating whether it was by weight or by volume and without giving yield percentage. [17] reported about yeasts that gave 8.5% (v/v) ethanol concentration from 30% sugars in the mash. This represents a yield of only 44.8% of the theory, which is very low. The ethanol concentration reached in this experiment was reasonable, but the yield was low, because the sugar concentration was too high. Ethanol concentrations and yields could have been better if lower sugar concentrations of about 20% (w/v) were used. The three strains of *Saccharomyces cerevisiae*tested in this study gave ethanol concentrations of 3% to 2.5% (w/v) representing yields less than 50% of the theory. This is very low and indicates that these yeast strains are of no importance for ethanol production. Being thermotolerant they could be of interest for SCP production. This has to be tested in further experiments.

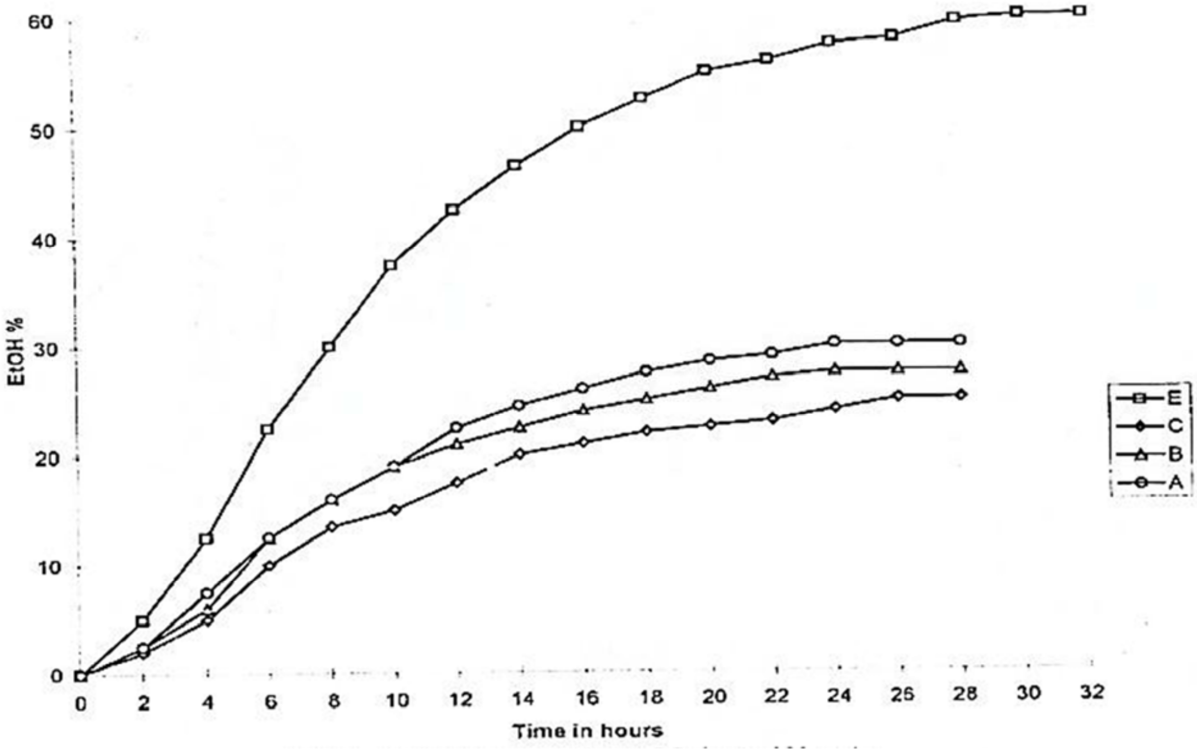

Figure 6. EthanolProduction of Selected Yeasts.

References

[1] Agab, M. A. Studies of some Aspects of Heat Resistance in Yeast. Ph.D. Thesis, Tu, Belfast. (1883).

[2] Hamad, S. H. Screening of Yeasts Associated with Food from the Sudan and their Possible Application for Single Cell Protein and Ethanol Production. Ph.d. thesis, Ing. Berlin. (1986).

[3] Abdelgadir, M. and Mustafa, M. M. Yeasts Causing Spoilage in Carbonated Beverages in the Sudan, J. Food Sci. Technol. (1978);10: 55-61.

[4] Baerwald, G. and Hamad, S. H. The Presence of Yeasts in Sudanese Can Sugar Factories, Zuckerind. (1984);109: 1014-1017.

[5] Barnett, J. A.; Payne, R. A.; and Yarrow, D. Yeasts: Characteristics and Identification. (ed.) Cambridge.(1983).

[6] Lodder, J. The Yeasts: Taxonomic Study. 2nd (ed.) north Holland, Publ. comp. Amsterdam, London. (1970).

[7] Barnett, J. A.; Payne, R. A.; and Yarrow, D. Yeasts Characteristics and Identification, 2nd (ed.) Cambridge University. Press London, New York, Melbourne.(1990).

[8] Kiss, I. Testing Methods in Food Microbiology. Amsterdam, Oxford, New York and Tokyo. (1984).

[9] Van Der Walt, J. P. and Yarrow, D. Methods for Isolation, Maintenance, Classification and Identification of Yeat. In N. J. W. Kregere Van Rig, ed. The yeast a taxonomic study. Elsevier science publisher. B. V. Amsterdam. (1984): 45-103.

[10] Agab, M. A. Isolation of Yeast Strains Suitable for Ethanol and Microbial Protein Production from Sugar Cane Molasses, M. Sc. Thesis, University of Khartoum, Faculty of Agriculture, Sudan.(1978).

[11] Caputi, A. Jr. and Wright, D. Collaborative Study of the Determination of Ethanol in Wine by Chemical Oxidation. J. AOAC. (1969); 52 (1): 85-91.

[12] Arthur, H. and Watson, K. Thermal Adaptation in Yeasts. J. Bacteriol. (1976); 128: 56-68.

[13] Einsele, A. Biomass from Higher N-Alkanes, Ico. Cif. (1983): 19-43.

[14] Barnett, J. A.; Payne, R. A.; and Yarrow, D. Yeasts Characteristics and Identification, 2nd (ed.) Cambridge University. Press London, New York, Melbourne. (1990).

[15] Hacking, A. J.; Taylor, L. W. F.; and Hanas, C. M. Selection of Yeast able to Produce Ethanol from Glucose at 40°C. appl. Microbial. Biotechnol. (1984); 19: 361-363.

[16] Ibrahim, H. H. A. Ethanol Production from Sugar Cane Molasses, M.Sc. thesis, University of Khartoum, Faculty of Agriculture, Sudan. (1993).

[17] Ali, I. A. M. Ehanol Production from Molasses by Yeast Fermentation. M.Sc. Thesis, University of Khartoum, Faculty of Agriculture. Sudan. (1987).