

**ISOLATION AND CHARACTERISATION OF YEASTS ASSOCIATED
WITH FRUITS, GRAINS AND BEVERAGES IN UGANDA**

By

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**A thesis submitted in fulfilment of the Degree of
Doctor of Philosophy of Makerere University.**

1994

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DECLARATION

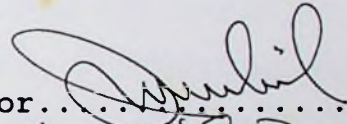
I, Cosmas Kafeero Kiwanuka, declare that The work presented in this thesis "Isolation and Characterisation of yeasts in Uganda" was done entirely by me from December 1991 to June 1994.

No part of this thesis has been submitted to any other University for a degree.

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DEDICATION

To GNNJ and all Ugandans in whose endeavours to maintain sustainable food production and plant resources this study is directly of importance.

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ABSTRACT

The study was undertaken during the period 1991-1994 to isolate and characterise yeasts associated with surfaces of fruits, grains and fermented products of the materials. The major aims of the study were to isolate and characterise yeast strains focusing on their habitats and to elucidate their physiological and biochemical potential through experiments and analysis.

Banana: The following yeast strains were isolated from banana in Masaka/Rakai: *Candida* Y40, *Candida* Y60, *Candida pulcherrima*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Hanseniaspora* Y68, *Hanseniaspora* Y20, *Saccharomyces* Y21, *Saccharomyces* Y12, *Schizosaccharomyces* Y26, *Cryptococcus* Y37, *Debaryomyces* Y13, *Schizosaccharomyces pombe*, *Saccharomyces pastorianus*, *Torulopsis* Y29, *Hansenula* Y49, *Candida* Y42, *Hansenula* Y62, *Lodderomyces elongisporus*, *Saccharomyces chevalieri*, *Dekkera bruxellensis*, *Saccharomyces* Y56, *Debaryomyces* Y10.

Tonto, Masaka/Rakai: *Candida* Y33, *Candida* Y6, *Dekkera bruxellensis*, *Hansenula* Y17, *Saccharomyces pastorianus*, *Saccharomyces ellipsoideus* Y68, *Hanseniaspora* Y68, *Saccharomyces* Y21, *Saccharomyces* Y12, *Schizosaccharomyces pombe*, *Schizosaccharomyces* Y26, *Cryptococcus* Y50, *Saccharomyces* Y25.

Muramba, Mbarara/Rukunguri: *Candida* Y42, *Hansenula* Y49, *Hansenula* Y62, *Hansenula* Y67, *Saccharomyces* Y30, *Saccharomyces* Y68, *Schizosaccharomyces* Y56, *Debaryomyces* Y40, *Debaryomyces* Y48, *Saccharomyces pastorianus*.

Amarwa from Jinja/Tororo/Mbale: *Candida* 2, *Candida* 11, *Hansenula* 2, *Hansenula* 32, *Schizosaccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schizosaccharomyces* 44M, *Schizosaccharomces* 44M, *Schizosaccharomyces* 44N, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Schizosaccheromyces* 44T.

Apart from the study of biodiversity, the study was also undertaken to study population density of yeasts responsible for certain fermentation processes. The fermented products of bananas, sorghum and millet were tonto, muramba, kwete and amarwa (ajon) in Masaka/Rakai, Mbarara/Rukungiri and Jinja/Tororo/Mbale.

In Masaka/Rakai the mean count of colony forming yeast units in tonto, the c.f.u. were $5.07 \times 10^7 \text{ ml}^{-1}$. In Mbarara/Rukungiri the counts for banana were 76.6 cm^{-1} ; Muramba $5 \times 10^7 \text{ ml}^{-1}$. In Jinja/Tororo/Mbale counts on banana were 2.5 cm^{-1} and amarwa $1.5 \times 10^7 \text{ ml}^{-1}$. Experiments on selected strains of yeasts show that some of the yeasts were preferentially anaerobic, yielding ethanol and CO_2 , potentially important in brewing and bread industry.

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Yield of ethanol under anaerobic conditions and biomass production under aerobic conditions were verified.

There was low pH change during controlled fermentation pH 4.0 to pH 3.8. The pH shifted significantly during uncontrolled fermentation typical of local brewing conditions. This indicated high level of acid production.

Ethanol is one of the products of the metabolism of glucose in tonto. The amount produced was directly related to the concentration of glucose in the medium. Some yeasts were intolerant of ethanol in their environment.

Yeasts have been discussed according to their cultural, morphological and physiological characteristics with the knowledge so obtained, it was possible and fruitful to consider the relationship of yeasts among themselves. Numerical analysis was applied to give a more objective system of grouping of the isolated and characterised yeasts. By studying clusters, it was possible to determine the characteristics the yeasts share as well as those in which they differ. Cluster analysis was used to identify the yeasts and their potential for biomass and ethanol yield and carbon dioxide yield.

CHAPTER ONE

1.0 INTRODUCTION

In Uganda, fermentation is as old as the history of its tribes. Each ethnic group has its history associated with the method used to produce the "beer" or "wine" or its "bread" or "pastes". This is shown by the important role the beverages play in cultural activities, such as marriages, funeral rituals, harvests, embalu (initiation ceremony) and other social activities in Western, Central, Eastern and Northern Uganda.

There is plenty of unwritten literature among the people of Uganda which gives details on fermentation procedures and processes. In general, two basic processes are used. The first involves use of millet (*Leusine corocana*), beer sorghum (*Sorghum bicolor*) and/or cassava, *Musa* cultivars, sorghum and water. The following is a detailed description of four common Uganda brews produced usually on a weekly basis at nearly every homestead.

1.2 TONTO (Central)

This beverages also called "mwenge bigere" is a major source of alcohol for Uganda waragi distilleries.

Tonto is a designation for sweetish, fermented juice, brown in colour, with a lot of debris or sediments. The juice is extracted from a variety of

banana cultivars grown along the shores and in the hinterland of Lake Victoria and on the slopes of Mountains, Rwenzori and Elgon.

The quality of tonto (in terms of taste, appearance and alcoholic content) depends on who prepares it, the variety of banana used, whether the fruits are peeled or unpeeled during juice extraction and the state of cleanliness of the containers used during fermentation. The "who" factor affects the quantity and quality of the juice extracted. Since squeezing is done manually or with the feet, the stronger these means are the more juice will be extracted.

If the squeezing is weak, juice extracted is limy and will not ferment properly. Each banana variety has a characteristic flavour and this affects the flavour of the tonto. Peelings add flavour and colour to the juice and provide yeast inoculum, hence, speed of fermentation. If the containers are heavily contaminated with bacteria and mould the tonto will deteriorate quickly and will have a musky smell. Roasted sorghum provides flavour and colour. Saccharification of starch could also take place and increase fermentable carbohydrate. No effort is made to prevent contamination. The containers used, such as dugouts (canoes), gourds, pots, jerrycans etc. are not sterile. No preservatives are added. The fermentation process begins almost immediately. The

containers are well insulated to conserve heat. Grass trash and banana leaves are used to cover the containers. The juice is left to ferment for 24 hours. The alcohol generated during the ensuing two or three days reached 7-8% measured by means of an alcoholometer. Alcohol is assimilated by bacteria and yeasts such as *Brettanomyces*, etc. to acetic acid, lactic acid and esters. The filtering is poor so that tonto never clears; is full of residues of grass trash, and sorghum. Tonto is consumed in local bars or distilled locally to produce "crude" gin called waragi or enguli.

The investigator intends, in future, to conduct experiments by using sterile conditions of brewing and incubation, controlled sugar content of banana extract, with *Hansenula*, *Saccharomyces* and *Schizosaccharomyces* both singly and in combination. The brew under these circumstances will be compared with the locally brewed tonto.

1.3 MURAMBA (Western)

There are a number of quite related preparations made from *Sorghum bicolor*. Sorghum is a staple food in many parts of tropical Africa, Purseglove (1988). The cereal is grown extensively in Kabale and Rukungiri districts in Uganda, where this account was recorded.

1.3.1 Unmelted Grain

After sorghum is harvested, it is threshed and winnowed. The clean grain may be ground and eaten in form of bread commonly called *Kancerate*, or made into a porridge called *enkumba*.

1.3.2 Malted Grain : *Obushera butoko* and *Muramba*

Cleaned sorghum is mixed with ash or *kashenda* to give dark brown colour to the grain. The grain is then soaked in water for 12 hours (approximately one night). The grain is then drained and placed in layers between banana leaves and allowed to germinate, in the house or outside in the yard, by periodic moistening for 4-5 days. Germination continues until the bud and radicle are about 2.5 cm long. During this time the starch undergoes saccharification to maltose and dextrins. Proteins are hydrolysed and vitamins are synthesized in the embryo. The radicles and plumules are removed. The grain is then placed under the sun on a clean mat to dry. The *kashende* (dried, dark brown sorghum grain) is now ready to make *obushera* and *muramba*.

The malted grain is ground into flower (green malt or "yeast") using a mill-stone or a mortar and pestle. The flour is mixed with water 50% (w/v) and boiled with great care. Over boiling spoils the porridge. At this time (stage) the mashed material is called *ekimyanya*. The *ekimyanya* is then mixed with

boiled, cooled water and allowed to stay for a night. The following day the porridge is called *obushera butoko*. This porridge is very sweet and nutritious as it contains sugars, starch, proteins, amino acids, vitamins and mineral salts.

The first stages in the preparation of *muramba* are more or less like those used in the preparation of *obushera butoko*. The diluted *obushera* is allowed to stay for several days (4-5 days). The fermented stuff is called *omushaze*. The *omushaze* is put into a container and re-boiled for more than eight hours, in a pot or *pipa* (metal barrel). The preparation is allowed to cool to room temperature (about 23°C). A starter mixture of old *muramba* with small amount of flour (green malt) about 6 mugs of 500 ml are added to each of 20 litres of the cooled preparation. The process is called *okuburira*. This preparation is left for a night. The following day a lot of green malt (flour), proportional to the original porridge is added. The process is called *okushembura*.

The period between the boiling of the *omushaze* and the *okushembura* is called *omutekye*. The mixture of *omushaze* and *esano* (green malt) is left to ferment for 24 hours. The fermentation is very vigorous. The result is *muramba*.

Other brewers continue increasing the amount of fermentable sugar by boiling again and adding more malted flour to form what is called *ekyanga*. The

ekyanga is cooled, diluted and more flour is added to form what is called *omwereera*. The *omwereera* is added to *omushemburo* to stay for a night. The mixture ferments very vigorously, like a volcano boiling out lava, as one brewer put it. The mixture boils over the container like lava oozing out of a crater. The result is a very intoxicating *muramba*.

1.4 AMARWA (Eastern/Northern)

In Masaka, Rakai, Mbarara, Ibanda and Rukungiri, amarwa brewing was typically associated with townships or Police Posts. Similar procedure is followed as for *muramba*, except what proportions of malted sorghum or millet to cassava or maize flour are added but 1: 4 is satisfactory. Approximately 25 litres of water are added for about every tin (10 kg) of grain. More millet malt is added at room temperature to make a paste. The mash is put into a pit, lined with banana leaves and well covered with the leaves. After 5-6 days the mash is brown colour. The roasted (roasted malt) paste is mixed with a lot of water in a barrel (about 200 litres) to which 5 mugs (2.5 litres) of millet flour (green malt) are added. Conversion or hydrolysis proceeds for 2 hours and fermentation for 2-6 days. Amarwa is ready for consumption while it is still actively fermenting. Ethanol is generally between 2-4%. The brew contains between 0.3 to 0.6% of acetic acid and variable quantities of esters.

Yeast present in the malt flour added afterwards together with species of *Mucor* and *Rhizopus* are responsible for hydrolysis and fermentation.

1.5 LITERATURE REVIEW

In all these processes of brewing, yeasts play a very important role.

The word "yeast" means fermentation. The French word *levule*, or the Latin word *levere* means to raise. The German *gist* or the Greek *zestos* refers to the bubbling of foam during the process of fermentation. The ancient world of the Greeks and Romans mixed fermenting juices with bread dough to manufacture raised or leavened bread.

The yeasts also grow and synthesize amino acid such as glycine, leucine, histidine, lysine, aspartic acid and glutamic acid, and vitamins (Platt, 1964). It is, therefore, essential that the products of fermentation not be clarified or filtered.

It follows, therefore, that indigenous fermentation products, either in form of tonto, muramba, or amarwa, or in form of porridge or bread or pastes, provide essential food values to the consumers in form of proteins, amino acid, vitamins and calories (Platt, 1964).

Millet and sorghum, together with cassava and beer bananas, because of their ability to resist drought or soil exhaustion, characteristic of many

parts of Uganda, these crops are crops *per excellence* for the dry regions usually with poor soils and poor rainfall. The survival of many people in Uganda depends on the resilience of these crops. These foods in their natural form are not very palatable. During the process of steeping, and fermentation processes, detoxication of bitter cassava takes place. In preparation of the grains and bananas certain undesirable odours and flavours are either destroyed or masked. There is plenty of potential associated with fermentation processes in Uganda, such as the following.

Single-cell-protein production: (SCP production) refers to the cells of yeasts grown for their protein content. Cells of these yeasts, especially species of *Candida* contain fats, vitamins and mineral salts. SCP products are used either for animal feeds or human food. They are potentially very important sources of amino acids, proteins, vitamins and mineral salts that can be prepared from otherwise inedible or unpalatable or low-quality waste materials (Platt, 1964).

Gasohol production

Gasohol is a mixture of ethanol and petrol. Theoretically ethanol can be produced from any carbohydrate source mentioned above, which can be converted into a form that can be metabolised by

yeasts.

There is paucity of written literature on yeasts in Uganda and a great demand for yeasts notwithstanding.

Yeasts serve many purposes. The selection of the most appropriate strains, species and varieties for special purposes, from the multiplicity of yeasts available, has been going on for a long time in industrialised countries (Hans, 1986). Weekly respiring, largely fermentative yeasts so called "bottom yeasts" or lager yeasts, used in production of beers such as the Uganda Nile Special, Bell Beer, etc. are on great demand, though imported at great expense on our foreign exchange.

The yeasts used for carbon dioxide and ethanol production, wine or ale making as baker's yeasts are largely "top yeasts". Yeasts are, therefore, commonly known as fermentative organisms because of their connection with brewing and baking (Kluyver, 1953; Phaff *et al*, 1966). Other yeasts are strongly oxidative and weekly fermentative (Kluyver, 1953; Phaff *et al*, 1966).

There are four distinct approaches to the problem of yeast characterisation. These include the study of cultural characteristics, morphological characteristics, sporulation (sexual) characteristics and physiological characteristics. Yeast characterisation is vital both in Applied Microbiology and in

Biotechnology. As a study, yeast characterisation was first established by Ress (1870) on pure cultures and brought to light the fact that yeasts are generally fermentative of carbohydrates. This led to the modern practise of employing cultural, morphological and physiological characteristics for identifying yeast species by physiological characteristics for identifying yeast species by Stelling-Dekker (1931) and Lodder (1952).

Guilliermond (1940) based his studies on sexual reproduction and suggested the phylogeny of the yeasts. Winge and Robberts (1958) carried out studies on yeast genetics and life cycles and laid down the modern concept of yeast physiology as subsequently expounded by Wickerham (1955).

Cultural or colony characteristics useful in the characterisation of yeasts include:

- (a) Liquid medium: sedimentation, formation of a pellicle or a ring islet; creeping up test tube walls; wrinkle formation.
- (b) On solid medium: the culture may be mucoid, butyrous or butter-like; friable, coherent; matted; rough or smooth; glistening surface; pigmented or not pigmented.

Morphological characteristics include: cell shape or cell size; whether cells reproduce vegetatively by budding, and whether budding is unipolar, bipolar or multipolar; whether the buds stick together to form

clusters or chains or both singles and clusters or chains; whether pseudomycelium or true mycelium is formed.

Physiological characteristics: these are very important in demarcating genera and species, (Lodder, 1970; Barnett and Parkhurst, 1984). Van der Walt *et al* (1984) remarks that because the definition or description of the yeast is neither clear cut nor precise, yeasts are identified by recognition of the overall similarities and affinities rather than of consistent and definitive characteristics. The author continues to say in absolute terms that no yeast can be characterised with confidence until a systematic study has been made of its cultural, morphological and physiological characteristics. "Let us call a yeast a yeast" says Flegel (1977).

The best characterisation system is built upon as many attributes as possible. Yeasts which share a large number of attributes would group or cluster together and form a "natural" group and such groups would separate from each other at points of rarity, that is, at combinations of attributes which never or very rarely occur (Hughes, 1988).

It follows from the above introduction that the following will guide the work:

1. To isolate yeast strains from fruits, grains and beverages collected from Uganda (Western, Central, Eastern and Northern regions).

2. To characterise the isolated yeast strains, using both conventional methods of van der Walt et al (1984), and Numerical Analysis.
3. Characterised and stocked yeast strains resulting in pure culture will form a potential source of yeast strains for further study and research projects such as developing mutants for improved yeast biosynthesis through crossing with existing useful yeast strains.
4. To provide consultancy and contractual research material for Uganda's breweries and bakeries.
5. To use the isolated and characterised yeast strains for further investigation following from this study, materials for publication.

CHAPTER TWO

2.1 MATERIALS AND METHODS

2.1.0 Materials

Materials used in this study were fruits, grains and beverages sampled from localities shown on maps in Appendix III.

2.1.1 Fruits

Bananas were all ripe fruits, local varieties which are in many ways similar to cooking bananas but are almost invariably used solely for beer making. All these varieties probably belong to the "AAA" genotype group of *Musa paradisica* (Purseglove, 1991).

The use of recently introduced varieties for beer (tonto) making is on the increase and some growers plant extensive areas to pure stands of these varieties for the sole purpose of beer production. The varieties include Bogoya (ABB), Kisubi (ABB), Sukari (ABB), Balukule and Kivuvu (ABB), groups of *Musa sapientum*). Bananas are produced in Masaka, Rakai, Mbarara, Ibanda, Rukungiri, Jinja, Tororo and Mbale.

Passion fruit, *Passiflora edulis*: passion fruits are cultivated or grow wild in all lacustrine regions of Lake Victoria. The pulp and seeds contain approximately: water 72.2%, protein 2.4%, fat 2.8%,

carbohydrate (sugar) 17.3%, mineral salts 1.2% and it is a good source of vitamin C. The juice is used for making soft drinks.

Pawpaw, *Carica papaya*: The edible portion of the flesh contains water 88%, sugar 10%, protein 0.5%, fat 0.1%, acids 0.1%, salts 0.6% and rich in vitamin A and some vitamin C.

Mango, *Mangifera indica*: The edible portion of ripe fruit is 60-75% by weight. Water 15%, protein 0.5%, sugar 10%, rich in vitamin A & B. The above figures are cited from Purseglove (1991).

2.1.2 Grains

Grains included the following: maize, (*Zea mays*), sorghum (*Sorghum bicolor*) and finger millet (*Leusine corocana*).

The beverages included tonto, kwete, muramba and amarwa.

Origins: Tonto from Central Uganda, and Western, Muramba from Kigezi, Amarwa/Ajon from the North-east of Uganda and in most urban areas, kwete from the east.

2.1.3 Chemicals

The following companies supplied the chemicals that were used: Griffin & George (G.G), Bishop Meadow Road, Loughborough Leice, England; Science and Education, 61Z-040-010D, England; Oxoid (Oxoid) Ltd Basingstoke, Hauts, England; Sigma Chemicals Co. 3500

Dekalb St. St. Louis, Missouri, USA (Sigma); The British Drug Houses (BDH) Lab. Chemicals Division, Poole, England; Difco Laboratories of Detroit (Michigan) (Difco). The chemicals used were of the Analytical Grade. These companies will be referred to when chemicals are mentioned in later sections of this chapter.

Substances used for preparing media were either extracts from natural sources available locally or from manufacturers. Yeast extract used for isolation cultural characterisation and maintenance of yeast cultures and for yeast characteristics in sexual reproduction, was an Oxoid product.

Malt extract (ME) used alone or in combination with yeast extract (YE) for isolation, cultural characteristics and maintenance of yeast cultures, yeast characterisation, formation of chlamydospores and for sexual reproduction was an Oxoid product. Peptone used for morphological characteristics was an Oxoid product.

Maize meal agar recommended by Bernhardt (1946), used for formation of pseudomycelium and true mycelium by yeasts was prepared as follows: 12.5 g yellow maize meal was stirred in 300 ml water and heated in a water bath at 60°C for one hour and then filtered through paper. The volume of the filtrate was made up to 300 ml and 3.8 g agar was added. The mixture was autoclaved for 15 minutes at 15 lbs overpressure. The hot

liquid was filtered through absorbent cotton wool, dispensed and sterilised for 15 minutes at 15 lbs overpressure.

Banana juice extract used in isolation and maintenance of yeasts was prepared as follows: 1000 g of peeled ripe banana (sukari) were soaked in 1000 ml of water for 10 hours at 4. The bananas were mashed and filtered through cloth, using a filter pump. The extract was autoclaved for one hour at 15 lbs pressure. The bananas were obtained locally.

Potato (*Solanum tuberosum*) extract 100 g washed, peeled and finely grated potatoes were soaked in 300 ml water for several hours in a cold room or refrigerator. The mass was filtered through cloth and autoclaved for 1 hour at 15 lbs pressure used for formation of pseudomycelium and true mycelium. The potatoes were obtained locally.

Vegetable juice (recommended by Mrak *et al*, 1942) for sporulation of yeasts was prepared using local materials as follows: 100 g per 100 ml of each of unpeeled carrots, beet roots, cucumber and potatoes were minced or grated finely and then mixed with water 50% (w/v). The mixture was autoclaved and expressed through cloth. The extract had a pH of about 5.

Agar: Bacteriological agar used for solid media was a G & G product.

Yeast autolysate, used in physiological characterisation of yeasts was prepared from Levure-

Séche Active (active dry yeast) a product of S.L.Lessafire Marcq, France.

Original weights of dry yeast and water 50% (w/v) were mixed in a flask. The mixture was incubated in a flask at 50-55°C for 3 days stirring occasionally. The mixture was boiled for 10 minutes and allowed to cool. The pH was adjusted to 6 (six) by the addition of N KOH. The supernatant was cleared by filtration with use of filter paper. The clear filtrate represented the full strength yeast autolysate.

Ammonium sulphate used in sporulation was a BDH product.

Carbohydrates

Bacto-dextrose used nearly in all media to supply energy to growing yeasts was an Oxoid product.

Galactose, sorbose, salicin, sucrose, maltose, melibiose, cellobiose, trehalose, lactose, raffinose, melezitose, inulin, starch used in analytical work (physiological characterisation) were all Oxoid products.

Alcohols and acids

Ethanol, glycerol, mannitol, lactate, succinate, citrate used in physiological characterisation were all Oxoid products.

Nitrogen compounds

Nitrate, nitrate, aspartic acid, arsparagine, glutamic acid, citrulline, ornithine, arginine used in physiological characterisation were all Oxoid and Difco products.

Vitamins: biotin, riboflavin, folic acid, niacin, thiamine, inositol used in physiological characterisation of yeasts were Oxoid and Difco products.

2.1.4 MEDIA

A. Media for isolation, cultural characterisation and maintenance of yeast cultures.

1. Yeast extract malt extract broth referred to as YM broth (Wickerham, 1951) was prepared as follows: 3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose were dissolved in 1 litre water. The pH of the medium was 5.5. For enrichment purposes the pH was adjusted to pH 3.7 with 1% hydrochloric acid. After dispensing, the medium was sterilised in an autoclave at 15 lbs pressure for 15 minutes.
2. Yeast extract - malt extract agar (YMA) was prepared by adding 20 g of agar to yeast extract - malt extract broth before sterilisation. The medium was sterilised at 15 lb pressure for 15 minutes.

3. Malt extract agar: 3 g malt extract was added to 20 g agar. After dispensing the medium was sterilised at 10 lbs pressure for 15 minutes.

B Media for morphological characteristics

1. 2% (w/v) glucose-yeast-extract-peptone agar. This medium was prepared by adding 2% (w/v) agar to 2% (w/v) glucose-yeast-extract peptone water. After dispensing, the medium was autoclaved for 15 minutes at 15 lbs.

2. Morphological agar:

I Characteristics of vegetative cells.

The composition of this medium is given in Table XXIX. To rehydrate the medium 35 g of Bacto-yeast morphology agar were suspended in one litre of cold distilled water. The suspension was heated to dissolve the ingredients completely. The solution was dispersed in tubes or flasks, depending on the needs and sterilised by autoclaving for 15 minutes at 15 lbs pressure. The final reaction of the medium was pH 5.6.

II Formation of pseudomycelium and true mycelium.

1. Maize meal agar: to 300 ml maize meal extract 3.8 g agar was added. The mixture was autoclaved for 15 minutes at 15 lbs pressure.
2. Potato-glucose agar: 230 ml of the potato extract was added to 770 ml of tap water

containing 20 g of glucose and 20 g agar. After this the medium was dispensed and sterilised for 15 minutes at 15 lbs pressure.

C Media for sexual characteristics

I Presporulation medium. This medium was made as follows: 10 ml beet root extract (100 g per 100 ml boiling water), 2 g dried yeast, 2.5 ml banana juice, 1 g calcium carbonate and 3 g. Water was added to give a final volume of 100 ml. After solution of the agar by steaming for 10 minutes, the medium was dispensed in tubes and autoclaved for 5 minutes at 15 lbs.

II Sporulation media:

1. Vegetative wedges: carrot, potato (irish), beet and cucumber wedges were used. The vegetables were thoroughly cleaned by washing. Long cylinders about 1 cm in diameter were cut from the vegetables with the aid of a cork borer. Wedges were prepared by cutting the cylinders obliquely. The wedges were rinsed with cold water and placed in suitable test tubes containing some water to prevent desiccation. The tubes were sterilised by autoclaving for 15 minutes at 10 lbs.

2. Vegetable juice agar (Mrak *et al*, 1942). Equal weights 300 g of washed, unpeeled carrots, beet roots, cucumber and potatoes (bought from Nakasero market, Kampala) were minced finely and mixed with 300 ml of water. The mixture was autoclaved and expressed through cloth. The extract has pH 5.5. To 300 ml the extract was added 20 g dried yeast and 20 g agar and the mixture made up to 1000 ml. The agar was dissolved by steaming. The medium was dispensed in tubes and autoclaved for 15 mins at 15 lbs. Another vegetable juice agar was used also recommended by Wickeham *et al* (1946). 14 g of agar was melted in 340 ml D.W. in another container (flask) was placed 350 ml of tomato juice (V8). This is canned vegetable juice, from supermarkets, containing a blend of tomatoes, carrot, celery, beets, parsely, lettace and spinach. To this tomato juice was added 5 g of moist dry yeast dispensed in 10 ml DW. Suspension was then heated for 10 min. The pH was adjusted until the cooked sample was 6.8. The hot medium was then poured into the hot agar solution, stirred and dispensed into tubes. Sterilisation was at 15 lbs, stirred and

dispensed into tubes. Slants were prepared as needed and sterilised for 10 minutes at 15 lbs.

3. Gorodkova agar (modified). To tap waster 1 litre the following were added: 19% (w/v) glucose and 20 g agar. The mixture was steamed until the agar dissolved and dispensed in tubes and autoclaved for 15 minutes at 15 lbs and slanted.
4. Fowell's acetate agar (Fowell, 1952). The following were dissolved in 1 litre D.W. 5 g sodium acetate trihydrate and adjusted to pH 6.2. Then 20 g agar were added and steamed to dissolve the agar, and dispensed in tubes, autoclaved for 15 minutes at 15 lbs and slanted.

Media for physiological characteristics of yeast

- I. Utilisation of carbon compounds: General media.
 1. Yeast autolysate (Lodder & Kreger van Rif, 1984).
 2. Fermentation basal medium (Wickerham, 1951).
4 g powdered yeast extract (a batch was used to support the growth of markedly vitamin-deficient, yeast e.g. *Kloeckera apiculata*) and 7.5 g peptone were dissolved in 1 litre D.W. sufficient bromothymol blue was added to give a sufficiently dense green colour.

Two ml aliquots were placed in 150 x 12 mm tubes carrying insert tubes. The tubes were sterilised by autoclaving for 15 mins at 15 lbs. On cooling the 1 ml concentrated, sterilised sugar solution were added aseptically.

3. Stock vitamin solutions (van der Walt & van Kerren, 1981); 0.2 g biotin, 0.2 mg folic acid, 200 mg inositol, 40 mg niacin, 20 mg riboflavin and 100 mg thiamine were dissolved in 1 litre D.W. and sterilised. The sterilised solution was dispensed in 10 ml aliquots and stored at 0°C.

II Utilisation of nitrogen compounds.

Media:

Only high grade (analytical grade) compounds were used as nitrogen sources in the nitrogen assimilation tests.

1. Assimilation in liquid medium (Wickerham, 1957). The liquid medium employed for testing the ability of yeasts to utilise the various nitrogen sources was similar in composition to that used in the tests on carbon assimilation. The composition is given in Table XXIX in the Appendix. The medium was prepared by dissolving 11.7 g Bacto-yeast, carbon base together with the requisite amount of the nitrogen source in 100 ml D.W. When the nitrogen source was a nitrate 0.78

g potassium nitrate was used. When it was a nitrite, 0.26 g sodium nitrite was used.

It was necessary to warm the water to effect complete solution, sterilised and stored in a refrigerator until required. The final medium was prepared by transferring aseptically 0.5 ml aliquots of the sterile medium into 4.5 ml amounts of sterile D.W. in 16 mm cotton plugged tubes which had been autoclaved for 15 mins at 15 lbs. Blank tubes were prepared by adding 0.5 ml of a filter sterilised basal solution. After the tubes had been shaken to mix the water and the medium were ready for use.

III Growth on media of high osmotic pressure.

Media

Growth on 50% and 60% (w/v) glucose-yeast extract agar. The two media were made up by dissolving 50 g and 60 g of glucose in 50 ml and 40 ml respectively of either yeast infusion or 1% yeast autolysate. The volume was noted and 3% (w/v) agar and dissolved by steaming or in a water bath. After dissolving the agar, medium was dispensed in 5 ml aliquots in (6 mm cotton-plugger tubes). The tubes were autoclaved for 15 minutes at 10 lbs and then slanted.

IV Grow at elevated temperatures.

Media

1. Growth at 37°C, 42°C.
 - (a) Liquid medium: A tube of the usual 2% glucose assimilation medium.
 - (b) On solid medium (Kreger van Rif, 1964).
YM agar, malt agar.
2. Maximum temperature for growth (van Uden & Farinha, 1978). Tubes containing glucose-yeast extract-peptone water are used.

V Acid production:

Medium

To 1 litre dilute (1:10) yeast autolysate 50 g glucose were added, and 5 g chalk (calcium carbonate) and 20 g agar were added, sterilise by autoclaving for 15 minutes at 15 lbs; cooled to 50°C, swirled gently to suspend the chalk evenly throughout the medium. 5 mls were distributed aseptically in tubes and slanted on a cold surface to avoid settling of the chalk.

VI Production of extracellular, amyloid compounds

Medium A: (Lodder & Kreger van Rif, 1984); 0.2% ammonium sulphate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulphate heptahydrate and 2% glucose were dissolved in D.W. pH adjusted to 4.5 using 1% HCl. An equal volume of 4% agar was prepared in D.W. The two separate portions of the media were autoclaved for 15 minutes at 10 lbs. The two portions were added

together aseptically while the agar was still liquified. The solutions were gently mixed to avoid, frothing. Petri dish plates were prepared containing 1 drop of yeast autolysate and 1 drop of vitamin solution. When slants were needed, then 5 ml aliquots were made in a cotton-plugged tubes and autoclaved as before.

Medium B: 10 g glucose and Bacto-yeast carbon base (see table XXIX in Appendix) were dissolved in 100 ml D.W. and sterilised, adding aseptically $\frac{1}{2}$ volume of the medium to $4\frac{1}{2}$ volumes of sterile liquified $2\frac{1}{2}\%$ agar. The solutions were mixed and plates or tubes, poured aseptically.

VII Pigment formation

Pigmentation. For production of pulcherrimin by *Candida pulcherrimina*, Beijerinck medium (as modified by van der Walt, 1952) was used. 4 g sucrose 2 g bacto-peptone 0.2 g potassium hydrate phosphate 0.005g magnesium sulphate heptahydrate, 1 ml yeast autolysate and 2 g agar were dissolved in 99 ml tap water. After autoclaving, 1 ml of a sterile 5% solution of ferric ammonium citrate (Merek's ferrie ammonium citricum fusc in lammelis) was added aseptically.

VIII Ester production

Media

1. YM agar
2. 20 ml 5% glucose solution in yeast autolysate 5% (w/v) glucose were dissolved in a yeast infusion; dispensed in cotton-plugged flasks; autoclaved for 15 minutes at 15 lbs.

2.1.5 Reagents A

For chemical determination of residual sugars.

- (a) Benedicts reagent (Browne & Zerban, 1941) (G & G): 173 g of sodium citrate and 100 g anhydrous sodium carbonate were dissolved in 800 ml D.W. and 17.3 g cupric sulphate pentahydrate were dissolved in 100 ml of D.W. mixing the solutions and making up to 1 litre.
- (b) Luff's solution (Schoorl, 1929). 50 g citric acid and 143 g anhydrous sodium carbonate were dissolved in 400 ml D.W., mixing with a solution of 25 g cupric sulphate pentahydrate in 100 ml D.W. made to 1 litre. The filtered solution keeps well.
- (c) Saturated, aqueous solution of sodium bicarbonate.
- (d) Hydrochloric acid.
- (e) Methylindol reagent: 0.5% solution of 3 methylindol in 90% ethanol.

- (f) Hydrochloric acid concentration (D 1.9); methyl orange indicator: 0.1% aqueous solution.
- (h) Glucose indicator papers. These were obtained under trade name "Glukotest", Boehringer and Sohne GmbH, Mannheim, W. Germany.
- (i) Methyl orange.

REAGENTS B: used in nitrogen assimilation tests, for positive nitrite reaction:

8 g sulphanilic acid were dissolved in 1 litre of 5 N acetic acid (1 part glacial acetic acid to 2.5 parts of water).

REAGENT C: For staining ascospores Schaeffer-Fultone modification of the Wirtz method (Schaeffer & Fulton, 1933) Malachite green safranine.

2.2.0 Methods

2.2.1 Introduction

"Experience has taught that no yeast can be characterised with confidence until a systematic study has been made of its cultural, morphological, sexual and physiological characteristics" (van der Walts *et al*, 1984).

The following criteria have been devised of the yeast and have been applied in the present study of yeast strains isolated from fruits, grains and beverages.

A Cultural characteristics

1. Growth in liquid media

2. Growth on solid media

B Morphological characteristics

Characteristics of the vegetative cells or asexual reproduction.

C Sexual characteristics

1. Formation of ^Sacid and ascospore

2. Interfertility

D Physiological characteristics

1. Utilisation of carbon compounds

(a) Fermentative utilisation of carbon compounds

(b) Assimilative utilisation of carbon compounds

2. Assimilative utilisation of nitrogen compounds

3. Growth in vitamin-free medium

4. Growth on media of high osmotic pressure

5. Growth at elevated temperatures

6. Acid production

7. Production of extra-cellular amyloid compounds

8. Pigment formation

9. Ester formation or production of aromatic compounds.

Before characterisation of the yeast strains^{can} be carried out using the above criteria, the yeast strain must be confirmed to be free from contamination. The plating of a single colony and the selection of a

second colony from the resulting replate practically guarantees of purity as a single-cell culture, provided that all the colonies on the replicate are of uniform type (van der Walt *et al*, 1984).

However, when dealing with ascogenous (sexual) materials, particularly with strains derived or isolated from natural substrates, it should be borne in mind that such yeast strains may be heterothallic. In such a case, the injudicious selection of single colonies after repeated plating of an actively sporulating culture not infrequently leads to the recovery of a single mating type and the loss of the actively sporulating diploid state. In this event, the isolation of both mating types must be undertaken (van der Walt *et al*, 1984).

Special techniques are described with each experiment, following the general isolation and characterisation, techniques for analysis and experiments in Chapter 4.

2.2.2 Selecting of samples and isolation of yeasts

Fruits: Ripe banana fruit samples were collected from the field as follows:

Ten random samples of ripe banana fruits were cut off from each of 40 clusters with sterile scissors and sterile gloves and placed immediately in clean, cellophane bags and tied, well labelled with date,

time, location, and variety of banana. The label for a particular sample, as an example, was as follows:

Date 6/2/92; time 10.00 a.m.; locality MR_{ij} where MR_j was a locality in Masaka, j stood for a village. So $MR_{11}B_1$ was a sample from Masaka, village Kasasa; the B_1 stood for a sample of bananas called Kisubi. MR_{2j} was a sample from Rakai. The general label for Masaka/Rakai was $MR_{ij}B_y$. The B_y stood for varieties of bananas.

The banana samples from the field were taken to the laboratory, Makerere Botany Department. When there was not enough time to start isolating the yeast strains, the samples were kept at 0°C in the refrigerator until the following day.

2.2.3 Isolation of yeasts from banana fruit

Yeasts were removed from the fruits by washing technique described by Warcup (1959). 40 ripe banana fruits were aseptically placed in a litre of sterile water in covered sterilised bowl. The bowl was manually shaken for 10 minutes to allow dislodging the yeast cells from the surface of the banana fruits into the water. The following technique was used in making serial dilutions. Ten ml of the fruit surface water extract in a sterilised cotton-plugged test tube was plated as follows: from the test tube containing 120 ml of the extract, 1 ml was plated on 5% malt extract agar, and YM agar, adjusted to an acid reaction, pH

3.5-4.0 for suppression of bacteria. One ml of the original surface extract was diluted to 1/10 and 1 ml was plated as above. From this new dilution one ml was diluted by a factor of 1/10 making the new dilution 1/100. This process was repeated until the dilution was 1/100,000. Plates were made at each dilution. The plates were incubated at 24°C, that is, at room temperature for up to 7 days. Colonies were counted at each dilution using a transparent squared paper for ease of counting. The dilution which gave a count of 30-100 colonies was used in subsequent isolation. The best dilution was 1/10,000. The method gave exact (mathematical) dilution ratio and ensured an approximately uniform degree of dilution at each stage. This enabled the worker to make statistical and probability analyses.

Six plates were made from each sample of ripe banana fruits. Four different media were used for the isolation and maintenance of yeast cultures. The media were banana-juice yeast extract agar (BYA), yeast-extract peptone-glucose agar (YPGA), nutrient agar-glucose (NAG), yeast extract malt extract agar (YMA).

For each medium, 25 ml each was dispensed onto sterile Petri dishes for cultivation and isolation of yeast strains and 10 ml each was dispensed into sterile McCartney tubes for agar slants for the maintenance of pure cultures.

From one or more of the dilutions, 1 ml samples were placed in sterile Petri dishes and dispersed with melted but cooled agar, at about 37°C, by shaking and rotating the plate before the agar solidified according to Warcup (1955b, 1957), Waksman (1927).

Cultures were incubated at room temperature (24°C) for 18 hours to 24 hours or 48 hours. Sometimes cultures were observed up to 7 days or one month, for slow-growing yeast strains. Since yeasts are unicellular organisms, it means that each colony developed or formed was considered to have originated from an individual yeast cell active on the surface of the fruit. The colonies were counted on the reverse side of the Petri dish, using a transparent squared paper.

Purification of the isolates was by replanting of colonies until the plates consisted of only one type of colony. Two replates were adequate to purify the strains. Pure cultures were maintained on YMA or MA and stored at 4°C.

The surface area of the banana was estimated using graph paper in mm, and colony counts were expressed as counts/cm².

Other fruits, such as mangoes, pineapples, pawpaws, passion fruits, were treated in the same way as the banana fruits, for yeast isolation.

2.2.4 Isolation of yeasts from grains

100 grams of millet were collected in sterilised jars, with tops on, and appropriately labelled, e.g. JT_{ij}MI for samples of millet from Jinja/Tororo where MI stood for millet. Yeasts were removed from the surface of the grain by washing 100 g in sterile water, as for bananas. Serial dilution plates were made and incubation was done as for bananas. 100 g of grain (millet) were weighed and colonies counted and expressed in terms of counts per gram.

Other grains (sorghum and maize) were treated in the same way.

2.2.5 Isolation of yeasts from beverages

Forty 75 ml sterilised bottles with screw tops were used to collect 40 samples of tonto. The fermenting beverage (24 hours of fermentation) was always available in the villages on special order from Masaka/Rakai.

Time was spent listening to narratives from people and observing how the tonto was prepared. Narratives were almost 100% identical for each of the brewers interviewed. The difference was either a canoe (dugout) was used to extract the juice using hands, or the bananas were squeezed with feet in shallow pit in the ground covered with banana leaves to prevent loss of juice. Samples from the field were brought to the laboratory. Bottles with their contents well labelled

were stored in the laboratory at 0° - 4°C . Ten ml of the fermenting tonto were plated as follows: ml of the tonto was plated onto BYA (banana juice yeast extract agar), yeast extract peptone-glucose agar and nutrient agar-glucose, yeast extract malt extract agar. Another 1 ml was taken and diluted to 1/10 and plated. From this new dilution, 1 ml was diluted to 1/100, and 1 ml plated as before. This dilution was again diluted to 1/1000, up to 1×10^{-6} . Plates were made at each dilution and incubated as usual. Dilutions of 1×10^{-6} gave the ideal colony count of 30-100 per Petri dish. Other beverages, *amarwa*, *kwete* and *muramba* were treated in the same way. Counts of colonies were expressed in number of colonies per ml of beverage.

2.3 Characterisation of yeasts isolated from fruits, grains and beverages

A total of 218 isolates were initially made from fruits, grains and beverages. These were reduced to 80 after preliminary diagnosis, and named Y_1 to Y_{80} . After further screening this number was reduced to 56 isolated yeast strains.

Two methods of yeast characterisation were used: Method one: The conventional method of yeast characterisation is according to van der Walt *et al* (1984), Barnett *et al* (1984), Phaff and Starmer (1987), Kreg va Rij (1984), Lodder (1970), and van der Walt and Johannen (1984).

Method two: Cluster Analysis or Procedure Cluster by computer according to Sneath (1956b) and Campbell (1974).

Conventional techniques of yeast strain characterisation

The main purpose of these techniques was to characterise and name, as far as possible, the yeast strain isolated. It was assumed that each yeast strain isolated had some cultural, morphological and physiological characteristics which one could use to identify and catalogue the strain for comparison with standard monographs of yeast taxonomy, like those mentioned above, e.g. van der Walt *et al* (1984). The method is based on cultural, morphological, sexual and physiological characteristics of yeasts.

Cultural characteristics

Practically all yeasts may be cultivated either on solid or in liquid media (broth). The characteristics of such cultures are observable with the naked eye and are used for diagnosis purposes (van der Walt *et al*, 1984). Two techniques are involved.

1. Growth in liquid medium: Each of the 56 yeast strains isolated was grown in liquid medium of malt extract broth (2%) and 2% of glucose yeast extract peptone water. The media 30 ml of each were placed in replicates in Erlenmeyer flasks,

well labelled with the strain, date, location and source. Appearance of growth in the flasks was recorded after 48 to 72 hours, then up to one month at 24°C.

The characteristics of growth were recorded in a table either for formation of a sediment, a ring, islets or pellicle. The pellicle whether dull or glistening, moist or dry; thick and tenacious or thin and brittle; the time taken for the pellicle formation; odours formed, if any, were recorded.

2. Growth on solid media: Agar was used as solidifying agent for the media. Agar melts completely at the temperature of boiling water and solidifies when cooled to about 40°C. It has no effect on yeast growth.

When an yeast culture was made on a solid medium, all the yeast cells produced either by budding or fission formed a colony visible to the naked eye. The colony had certain characteristics, such as texture, size, shape, nature of margin, colour and elevation.

An axenic culture (Greek axenos means no guest friend; no stranger not contaminated by or associated with any foreign organism) contains only one kind of yeast strain. As a rule only pure cultures were used.

Streaking an agar plate: Using a flame-sterilised loop a bit of inoculum was picked up and aseptically (in an inoculation chamber) streaked or passed across the agar plate. The media used were malt agar 2% (w/w) and glucose-yeast extract-peptone agar (2%).

The plates were incubated at 24°C. Appearance of the streak culture was recorded after 24 to 72 hours, made again after 4 weeks.

On morphology agar, colonies were described after 6 to 8 days incubated at 24°C.

Results were recorded as follows: growth was either mucoid, butyrous (butter-like), friable, coherent, matted. Some colonies existed as rough, smooth or glistening. Growth was either strongly pigmented by production of pigments. The pigment either required the presence of iron in the medium or was independent of iron, in which case the pigments were carotenoids. The former was due to a pigment called pulcherrimin.

The pigments were recorded as red, yellow, cream, brown, pink or white or pulcherrimin.

Morphological characteristics

Characteristics of vegetative reproduction or sexual reproduction occur in yeasts either by fission, budding or by a combination of these two processes.

Methods

Materials from a young, actively growing pure culture were inoculated into 30 ml malt extract broth (2%) and glucose-yeast extract-peptone water in 100 ml Erlenmeyer flasks. After 24 to 72 hours incubation at 24°C, the cultures were examined microscopically. A portion of the culture was aseptically transferred from the culture onto a slide with a drop of sterile water, using a flame sterilised loop. The smear was covered with a coverslip and examined microscopically under medium and high power. The length and width of the yeast cells were measured using a calibrated eyepiece micrometer scale and the extreme values obtained from the measurements of 20 cells were recorded. The cultural characteristics were also recorded. The flasks were then set aside at 24°C and again described 4 weeks after inoculation.

When employing morphorology agar, use was made of the Dalmau method of plate culture (Wickerham, 1951). Freshly poured plates were set aside for 24 to 48 hours in order to allow the surface to dry. A single streak inoculation was made, say as from the relative positions of 10 o'clock to 2 o'clock, of the clock. The inoculation was light, from a fresh slant culture. In addition to the single streak inoculation described above, two point inoculations were made near the other side of the plate as at positions 4 o'clock and 8 o'clock. A central section of the streak and one of

the point inoculation were covered with sterile coverslips. The plates were incubated at 24°C. After 3 to 4 days cells at the very edge of one of the single uncovered point inoculation colonies were examined for size, shape and arrangement of cells. The same plates served for the detection of mycelium and pseudomycelium as well as for description of the colonies.

Observations were recorded as follows:

1. Shape: the cells were recorded as spheroidal, sub-globose, ellipsoidal, ovoidal, globose, cylindrical, rectangular, elongated, bottle-shaped.

Size: the size of cells was recorded as length X width, in micrometers.

2. Formation of pseudomycelium and true mycelium: if a yeast reproduces exclusively by budding the mature bud may either detach itself immediately or may either adhere to the mother cell and eventually give rise to clusters or chains of cells. The tendency to remain in aggregates may result in the formation of pseudomycelium. The term pseudomycelium indicates the formation of a filamentous structure consisting of cells which arise exclusively by budding (van der Walt, 1984).

If a yeast which reproduces solely by fission forms filamentous cells, these constitute true

mycelium, with septa. When mycelium breaks apart, one-celled arthrospores are formed.

The study of Pseudomycelium and true Mycelium

For the study of pseudomycelium and mycelium formation, maize meal agar, potato (irish) glucose agar or morphology agar were used, using slide culture method or spot-plate technique (Wickerham, 1951).

Method

Slide culture procedure: a Petri dish, containing a U-shaped glass-rod support on which two glass slides were placed, was sterilised by dry heat at 160°C for 2 hours in an oven. Maize meal - and potato-glucose-agar were melted and poured into the second Petri dish. The glass slides were quickly removed from the glass rod with a flame-sterilised pair of tweezers or tongues, and were dipped into the agar after which they were replaced on the glass support. After solidification of the agar on the slides the yeast was lightly inoculated in three lines along each slide and a sterile coverslip was placed over part of the lines. Some 3 ml of sterile water was poured into the Petri dish, or strips of sterile moistened filter paper were used to prevent the agar from drying out. The culture was then incubated at 24°C for 5 days.

Results were recorded as: pseudomycelium or true mycelium present or absent or the yeast a single cell.

Formation of chlamydo spores: the chlamydo spores have been defined as thick-walled, nondeciduous intercalary or terminal asexual spores.

Method: The same as for pseudomycelium formation.

Sexual characteristics

Yeasts may be either homothallic or heterothallic. Homothallic yeasts are self-fertile while heterothallic ones are self-sterile. Heterothallic yeasts require another mating type to effect fertilisation. In homothallic yeasts ascospore formation is initiated by reduction division of the diploid nucleus within a vegetative cell and the latter is directly converted into an ascus (van der Walt, 1984).

If heterothallism is operative, cells or ascospores of both mating types may conjugate directly to form a zygote which undergoes meiosis to produce asci. The number of ascospores per ascus may vary from one to many. The shape of the ascospores also varies considerably.

Ascospore formation is quite a complex process involving *inter alia* such factors as the nutritional conditions of the culture, the composition and pH of the sporulation medium, temperature and the degree of aeration (Phaff and Mrak, 1949).

As considerable difficulty is sometimes encountered in inducing poorly nourished cells to form ascospores, it is necessary to culture some yeast strains on a rich presporulation medium before these are transferred to the sporulation media.

In testing for the ability to form ascospores it is necessary to use a wide range of media. For most yeast strains ambient room temperatures, e.g. 24°C are quite suitable for the sporulation of yeasts. Some yeast species are slow in forming ascospores and cultures may require 3 to 6 weeks before the ascospores are formed (van der Walt, 1984).

Methods

The culture to be studied was first brought to a state of active and optimal nutrition by sub-culturing on a presporulation medium or directly on yeast malt agar or malt for 24 to 48 hours, at 24°C. The sporulation media described earlier were then lightly inoculated with the culture. The inoculated sporulation media were then incubated at 24°C for 72 hours before being examined microscopically for the first time. Material that had not sporulated was then maintained at room temperature and examined at weekly intervals for at least 6 weeks.

Ascospore formation was verified by staining the heat-fixed preparations.

Staining procedure

Schaeffer-Fulton's modification of the Wirtz method (Schaeffer & Fulton, 1933) was used. Heat-fixed slide preparations of yeast were flooded with 5% aqueous malachite green for 60 seconds and heated to steaming 4 times, care being taken not to crack the glass slide. The excess stain was run off under running water for about half a minute. The preparation was then counter stained with 0.5% safranin for about 30 seconds. The mature ascospores stained blue-green and the vegetative cells red.

Results were recorded as + (ascospores formed) or - (ascospores not formed) and also in terms of shape of ascospores as spheroidal, ellipsoidal or cylindrical.

Isolation of mating types of heterothallic haploid yeast species (Winge and Laustsen, 1939)

Method

A species that produced few or no ascospores when isolates were tested individually on sporulation media, were tested in mixtures of four strains in order to enhance chances of success in obtaining mating primitive (wild) species of yeasts. To make mixtures, approximately equal amounts of actively growing cells were mixed in the centre of a slant of sporulation medium and the mixture was spread out. Observations were made for the presence of ascospores

after 6 days and then weekly over a period of three weeks. If a sexual reaction was observed which was in excess of the reaction that was seen to occur in the individual strains, the four strains of the reacting group were mixed in six pairs to determine which of these strains were crossing mating. Details of these procedures are given by van der Walt (1984).

Physiological characteristics of yeast strains isolated from fruits, grains and beverages in Uganda

In general, the characteristics which have proved most useful in yeast characterisation are those associated with the utilisation of carbon and nitrogen sources; growth factor requirements; the fermentation of typical characteristic metabolites, such as esters (van der Walt *et al*, 1984).

I Utilisation of carbon compounds: - yeast developing on a carbohydrate source must either be able to ferment it or to utilise it by respiration. It is customary in characterisation of yeasts to refer to the first process as "fermentation" and to the second as "assimilation" (van der Walt *et al*, 1984).

(a) Fermentative utilisation of carbon compounds: the range of carbohydrates employed in the fermentation tests included Monosaccharides: D-glucose, D-galactose; Disaccharides: sucrose,

maltose, cellobiose, trehalose, lactose and melibiose; Trisaccharides: raffinose, melezitose; Polysaccharides: inulin and soluble starch.

Fermentation of raffinose is associated with formation of residual sugars, so that either $\frac{1}{2}$ or $\frac{1}{3}$ of complete molecule is fermented. Normally during the fermentation of raffinose for $\frac{1}{2}$ of the molecule, the fructose fraction is fermented with the formation of residual melibiose by yeasts. Raffinose may also be fermented for $\frac{1}{3}$ with formation of residual sucrose. Under these circumstances only the galactose fraction is fermented. The fermentation of $\frac{1}{3}$ of the raffinose molecule is effected by yeasts which are capable of hydrolysing the raffinose molecule completely. This fermentation of $\frac{1}{3}$ of the raffinose molecule is associated with the ability to ferment sucrose and also with the ability to ferment $\frac{1}{2}$ the melibiose molecule. An indication of the degree to which the raffinose molecule is fermented by a yeast strain may, therefore, be obtained by inspection of its ability to utilise raffinose, galactose, sucrose, and melibiose. The residual carbohydrate in the case of incomplete fermentation of raffinose was determined chemically according to Wickerham *et al* (1951).

Methods

Only high grade sugars of certified purity were used, (Oxoid, Sigma for example). The fermentation of

all sugars was tested in 2% solutions, except in the case of raffinose where a 4% solution was normally used.

The sugars were dissolved in distilled water. Six ml aliquots were dispensed into cotton-plugged 150x12 mm tubes, carrying inserts tubes approximately 50x6 mm. A basal medium consisting of peptone (7.5 g) and yeast extract (4.5 g) per litre containing bromothymol blue was prepared. Two mls aliquots of the basal medium with 1 ml of the indicator were pipetted into the tubes. The tubes were sterilised for 15 minutes at 15 lbs pressure. The fermentation media were inoculated in an inoculation chamber with about 0.1 ml of a suspension of yeast cells made by suspending the growth of a 24 to 48 hours yeast malt or malt agar culture in 4.5 ml sterile water. The tubes were incubated at 24°C. The tubes were regularly shaken and observed for percent gas in the inserts and for change in colour of the indicator for acid production over a period of 24 days. The results were recorded as (+) for a reaction and (-) for absence of reaction or response by the yeast, that is fermented or not fermented respectively. Other details were whether fermentation was rapid or slow or delayed, and recorded as +, S, D respectively.

Change of colour indicated acid production. When raffinose was fermented, it was ascertained whether $\frac{1}{2}$ or $\frac{3}{4}$ or complete molecule was fermented with the

formation of residual melibiose, sucrose, or galactose, an indicator which was obtained from the table below.

Fermentation of raffinose

Fermentation of				Assimilation of				Presumed degree of fermentation of the raffinose
Ra	Me	Su	Ga	Ra	Me	Su	Ga	
+	+	+	+	+	+	+	+	Complete
+	+ ₁ S	+	-	+	+S	+	-	% molecule (residual galactose)
+	-	+	+	+	-	+	+	% molecule (residual melibiose)
+	-	+	-	+	-	+	-	% molecule (residual melibiose)
+	+	-	+	+	+	-	+	% molecule (residual sucrose)

Key: Ra = raffinose, Me = melibiose, Su = sucrose
Ga = galactose, S = slow

Method : chemical determination of residual sugars

The procedure requires only one fermentation tube with 2% raffinose per yeast strain. The procedure is based on three simple chemical colour reactions. The presence of residual reducing sugar in the fermented liquid is determined directly with Luff's or Benedict's reagent. Aliquots of the fermented liquid were then hydrolysed and tested specifically for glucose with glucose-oxidase indicator paper, and for fructose with methylindol reagent. The method was as

follows: A Durham fermentation tube containing 2% raffinose in 1% peptone water was incubated with the yeast under test and incubated until fermentation was complete and no further carbon dioxide was evolved.

1 Determination of reducing sugar

One ml of the fermented liquid was mixed with 5 ml of Luff's reagent or Benedict's reagent in a test tube and boiled for 1 minute over an open flame. If reducing sugar was present, the clear blue liquid turned green and turbid. After a while a yellow or red deposit of cuprous oxide was formed. If reducing sugars were absent the liquid remained blue.

2 Determination of glucose

Ten drops of the fermented liquid were mixed with 1 ml 10% hydrochloric acid in a test tube and heated for 4 minutes in a boiling water bath to effect the hydrolysis of residual sugar. Then 1 drop of methyl orange indicator was added and the solution was neutralised by adding a saturated solution of sodium bicarbonate drop by drop. Glucose indicator paper strips were dipped into the cooled neutral solution and exposed to the air. The strips turned blue if glucose was present, but remained yellow if this sugar was absent.

3 Determination of fructose

Ten drops of the fermented liquid were mixed with 0.2 ml methylindol reagent and 3 ml concentrated HCl. If fructose was present the light orange solution

turned deep red after a few minutes. The degree to which raffinose was fermented was determined from the table below.

Medium	Time	Color	Reaction
1	1	-	0 (initial)
2	2	-	0 (initial)
3	3	-	0 (initial)
4	4	-	0 (initial)

(b) Oxidation or reduction of carbon
 as in the case with raffinose, this was all in
 of oxygen and carbon dioxide. The reaction
 was rapid and the color changed from red to
 yellow. A small amount of gas was evolved
 and the liquid became slightly turbid. The
 reaction was complete in about 10 minutes.
 The color of the liquid was red at first, but
 after 10 minutes it had become yellow. The
 reaction was complete in about 10 minutes.
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Determination of the degree to which raffinose is fermented

Reducing sugar	Glucose	Fructose	Fermentation of raffinose
+	+	-	¼ (residual melibiose)
-	+	-	½ (residual sucrose)
+	-	-	¾ (residual galactose)
-	-	-	Complete

(b) Oxidative or assimilative utilisation of carbon compounds

As in the case with fermentative characteristics, assimilatory characteristics are employed for demarcation of genera and for speciation purposes.

A number of growth tests have been devised to determine the ability or inability of yeasts to utilise carbon compounds (Wickerham and Burton, 1948). In the test, the compound was added to a complete liquid basal medium in which the compound served as the sole source of carbon. A test run over several weeks. It permitted the adaptation of the yeast to certain compounds. Erroneous results were avoided by the use of a blank consisting of the inoculation basal medium devoid of any carbon source. The cultures were agitated to improve on aeration.

Wickerham and Burton (1948) after screening 70 compounds using 100 yeasts strains representative of 22 genera selected 30 compounds which held most promise for yeast characterisation purposes.

In this study the following compounds were used.

Hexose sugars and their derivatives: D-glucose,
D-galactose, L-sorbose, α-methyl-D-glucoside, salicin.

Disaccharides: sucrose, maltose, melibiose,
cellobiose, trehalose and lactose.

Trisaccharides: raffinose, melezitose

Polysaccharides: inulin and soluble starch

Pentose sugars: D-xylose, D-arabinose and L-rhamnose.

Alcohols: ethanol, glycerol, ribitol, galactitol,
D-mannitol, D-glucitol, inositol

Acids: DL-lactic acid, succinic acid and citric acid.

Methods

High grade, standardised products were used. Preparation of the inoculation medium: The nitrogen basal medium for carbon assimilation tests in liquid medium was used. The composition of the basal medium is given in Table XXIX in the Appendix.

The medium was prepared by dissolving 6.7 g Bacto-yeast nitrogen base and the appropriate amount of the carbon compound equivalent to glucose, that is, containing the same amount as 5 g glucose in 100 ml distilled water. When raffinose was the carbon source

it was used at twice this concentration. At times it was necessary to warm the mixture to effect complete solution. When organic acids or their salts were used as carbon source, the pH of the solution was adjusted to 5.0 by the addition of sodium hydroxide pellets.

When soluble starch or inulin was used as carbon source, it was dissolved in distilled water by gentle heating over an open flame. The solutions containing 0.5% of the carbohydrate were pipetted in 4.5 ml distilled water in cotton-plugged 150 x 12 mm tubes. 2 ml of the Bacto-yeast nitrogen base were added. The tubes were autoclaved for 10 minutes at 10 lbs and the tubes were ready for use.

Preparation of the inoculum

Before proceeding with the test the strain to be tested had first to be brought to a state of active growth. This was effected by transferring the strain twice on yeast malt agar at 24°C at three day intervals. Cell suspensions provided the material employed for the inoculation of the media used in the assimilation tests. A loop-full of the yeast strain was aseptically suspended in 10 ml sterile distilled water. 5 ml of the inoculum in 150 x 12 mm tubes was diluted until black lines approximately $\frac{1}{4}$ mm wide drawn on white cardboard became visible through the

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tubes as dark bands. Each tube in the assimilation tests received 0.1 ml of the diluted inoculum, using sterile pipettes.

As there were 56 yeast strains to deal with, and 28 carbon compounds, giving a total of 1,568 tubes, great care was taken in labelling each tube with the carbon source and the strain used. Tubes were set in racks to make observations easy.

After inoculation the tubes were incubated at 24°C, room temperature. The tubes were agitated manually to improve on aeration. Observations were made at weekly intervals over 4 weeks. The first observation consisted of recording the amounts of growth; also at this time tubes were observed for pellicle formation. The degree to which the various carbon compounds were utilised was determined by placing the tubes which had been vigorously shaken to disperse all yeast growth, against a white card bearing lines approximately $\frac{1}{4}$ mm wide drawn with black Indian ink. If growth in the tubes completely obliterated the lines, it was recorded 3+; if the lines appeared as diffuse bands, the growth was rated as 2+; if the bands were distinguishable as such but indistinct edges it was recorded as 1+; while the absence of growth was indicated as -: A 3+ or 2+ reaction within 4 weeks was considered to be positive

and a 1+ reaction as very weak or negative. At the final reading, tubes were tested for presence of amyloid compounds.

II Assimilative utilisation of nitrogen compounds

Since nitrogen metabolism is a basic feature of growth, the ability or inability to utilise different sources of nitrogen can be made use of in characterisation of yeasts (van der Walt *et al*, 1984). The ability to utilise potassium nitrate, sodium nitrite and specific amino acids, has proved a most useful taxonomic characteristic.

For yeast strains characterisation purposes the ability to utilise a nitrogen source is assessed by determining growth response under aerobic conditions.

Methods

Only high grade, analytical grade compounds, were used as nitrogen sources in the nitrogen assimilation tests. Liquid media were used.

(a) Utilisation of nitrate

Basal medium: 11.7 g Bacto-yeast carbon base (Table XXIX) together with 0.78 g of potassium nitrate (KNO_3) were dissolved in 100 ml distilled water, warming a little to effect complete solution of the constituents. 0.5 ml of the basal medium was dispensed into 4.5 ml distilled water in 150 x 12 mm

cotton-plugged tubes, which were then autoclaved for 15 minutes at 15 lbs. Blank tubes were prepared by putting 0.5 ml of basal medium into 4.5 ml distilled water in cotton-plugged tubes, same size as those for the test, and autoclaved similarly. The tubes were then ready for inoculation. The medium was inoculated in the same way as the carbon assimilation tubes using cell suspension in sterile distilled water. The tubes were incubated at 24°C. The tubes were observed after 7 days. Growth was similarly assessed as for carbon assimilation with 3+, 2+, 1+ or negative growth. Nitrate assimilation was confirmed by chemical test for nitrite.

Reagent: 8 g sulphanilic acid in 1 litre of 4 N acetic acid (1 part of glacial acetic acid to 2.5 parts of distilled water). A few drops of each of the reagent were put in nitrate cultures. If a distinct pink colour formed the presence of nitrite was indicated. Absence of nitrite in the presence of good growth indicated complete consumption of the nitrate.

(b) Utilisation of nitrite

Testing the assimilation of nitrite may involve toxic effects due to nitrous acid formation, if the pH of the medium is below 6. So tests were made at low concentration of nitrite.

Medium: 11.7 g Bacto-yeast carbon base were dissolved with 0.26 g sodium nitrite in 100 ml

distilled water. The rest of the procedure was as for carbon assimilation that is, sterilization and preparation of inoculum, inoculation and recording of results.

(c) Utilisation of amino acids

11.7 g Bacto-yeast carbon base together with 0.64 g of the amino acid were dissolved in 100 ml distilled water. The rest of the procedure was as for the carbon assimilation.

III Growth in vitamin - free medium

In view of the essential role of vitamins in yeast metabolism, vitamins requirements provide useful diagnostic criteria in yeast characterisation (van der Walt *et al*, 1984).

The following vitamins were used: biotin, riboflavin, folic acid, niacin, thiamine hydrochloride and inositol.

Method

The composition of the medium is given in Table XXIX. The medium was prepared by dissolving 16.7 g Bacto-vitamin free yeast base in 100 ml distilled water. The final solution was prepared by pipetting 0.5 ml of the basal medium into 4.5 ml distilled water in 150 x 12 mm cotton-plugged tubes.

Vitamins were added as follows: folic acid 2 µg, inositol 10 gm, niacin 400 µg, riboflavin 200 µg, thiamine hydrochloride 400 µg. The solution was sterilised by autoclaving for 10 minutes at 10 lbs pressure. The tubes were then ready for inoculation. The media were inoculated in the same way as the carbon assimilation tubes. The tubes were incubated at 24°C. The tubes were observed after 7 days and the results recorded as 3+, 2+, 1+ or negative growth.

IV Growth on media of high osmotic pressure

Yeast isolated from substrate with high sugar content are generally resistant to high osmotic pressures. Some osmotophilic species also show appreciable alcohol tolerance an interesting characteristic for brewers. The ability to grow on high concentrations of sugar was tested by growth on agar media containing 50% and 60% (w/w) glucose media (van der Walt *et al*, 1984).

Method

The two media were made up by dissolving 50 g and 60 g of glucose in 50 ml and 40 ml respectively of 1% yeast autolysate. The media were dispensed in 4.5 ml aliquots in 150 x 12 mm cotton-plugged tubes and sterilised by autoclaving for 10 minutes at 10 lbs pressure. The tubes were then ready for inoculation. The tubes were incubated at 24°C for 4 weeks. Growth

amounting to 1+ after 20 to 24 days incubation at 24°C was recorded as weak, at 2+ cell density was recorded as moderate and 3+ as good.

V Growth at elevated temperatures

Temperatures between 20 and 28°C are optimal for the growth of most yeast species (van der Walt *et al*, 1984). Taxonomically it is useful to determine whether or not yeasts are capable of growth at 30°C or above, especially for yeasts associated with fermenting beverages, where temperatures may rise to over 42°C.

Growth at elevated temperatures was determined in liquid media.

Method

(a) Growth at 30°C: a tube of the usual glucose assimilation medium was inoculated as usual and incubated at 30°C. Growth was recorded in the usual way after 7 and 24 days. Results were observed in the same manner given before.

(b) Growth at 37°C and at 42°C: The same procedure was followed as for growth at 30°C except that the culture were incubated at 37°C and 42°C. Incubation, observation and recording of results were done as usual.

VI Production of extracellular amyloid compounds

Aschner *et al* (1945) studied the production of extracellular amyloid or starch-like materials by encapsulated yeasts. They found that under suitable cultural conditions several yeast strains produced extracellular polysaccharides which gave a blue colour with iodine solution. One of the conditions was that the pH of the medium was maintained below 5.

Methods

Medium (Lodder & Kregar van Rij, 1984): 0.2% ammonium sulphate, 0.2% potassium dihydrogen phosphate, 0.1% magnesium sulphate heptahydrate and 2% glucose were dissolved in 100 ml distilled water, pH adjusted to 4.5. Agar 4% was prepared in distilled water. The two separate portions of the media were autoclaved for 15 minutes at 10 lb pressure. The two portions were added together aseptically while the agar was still liquified. Petri dishes were prepared containing 1 drop of yeast autolysate and a drop of concentrated vitamin solution.

Lugol's iodine solution (Gram's modification) 1g iodine and 2 g potassium iodide were dissolved in 300 ml distilled water. Plates were inoculated by streaking with the test yeast, incubated at 24°C. After incubation for 1-2 weeks the plates were flooded with dilute iodine solution. Strains producing

amyloid material showed colour reactions varying from blue to purple or green. The blue colour indicated amyloid material on yeast walls.

VII Pigment formation

The formation of distinctive pigments can be used for differentiating yeast strains. Three main types of pigments may be distinguished: carotenoids, pulcherrimin and ribosylaminoimidazole. Carotenoid pigments vary from shades of red to yellow which are detected visually. Not all red yeast colonies owe their colour to carotenoid substances. Beijerinck (1918) reported the formation of a red pigment in the yeast *Candida pulcherrimina* and pointed out that the formation of the pigment occurred only in presence of iron.

Method

Pigmentation is normally observed in growth on the media employed for determining the cultural properties of yeast strains. However, for the production of pulcherrimin iron must be added to the medium. The medium was prepared by dissolving 4 g glucose, 2 g Bacto-peptone, 0.2 g potassium dihydrogen phosphate, 0.05 g magnesium sulphate heptahydrate, 1 ml yeast autolysate and 2 g agar in 99 ml tap water. After autoclaving 1 ml of sterile 5% solution of ferric ammonium citrate was added aseptically. Plates

were prepared as usual, inoculated and incubated at 24°C for 1 week and observed for the production of pigment in the medium.

VIII Ester production

When fermentative yeast produces esters in sufficient amounts to be easily detected by odour, this property can be used for the characterisation of yeast species.

Method

A plate culture of the yeast strain under test was prepared on yeast malt agar and incubated at 24°C. The culture was judged by smell after 24 to 48 hours. The results were recorded as either positive for ester production or negative.

CHAPTER THREE

3.0 RESULTS

3.1 Characterization of yeast strains isolated from fruits, grains and beverages

Initially eighty one yeast strains were isolated and named Y1 to Y81. After diagnosis these were reduced to fifty six strains. The yeast strains were isolated from fruits, grains and beverages in Uganda. The yeast strains were characterised using monographs (van der Walt *et al*, 1984). The characterisation tests accounted for 9 cultural characteristics, 10 morphological characteristics 10 primary physiological tests; and 6 confirmatory tests. This gave a total of 25 characterisations. The total number of variables involved was 18 cultural, 11 morphological, 6 fermentation, 37 for assimilation, 3 of temperature, 1 osmotic pressure, 2 of vitamin and amylose production, giving a total of 79 variables. The results of yeast strain characterisation were summarised in Table I.

RESULTS

Table 1 Yeast characterisation

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y50	MR _{ij} , Fruits	Culture: wrinkled pellicle; colourless Morphology: pseudomycelium; Physiology: Ferments well= D-glucose, raffinose, sucrose; assimilates; D-glucose, cellobiose; D-glucosamine; does not produce amyloid compounds.	Can Y50
Y33	MR _{ij} , Tonto	Culture: same as for Y50 Morphology: budding; true mycelium, producing clusters cells ovoid to more or less cylindrical. Physiology: Ferments D-glucose, lactose, Sucrose, raffinose; weakly assimilates glucose; delayed assimilation of sorbose; assimilates glycerol mannitol; nitrate positive; assimilates Asporagire.	Can
Y6	MR _{ij} , Tonto	Culture: Same as for Y50 Morphology: Cells 5-9 x 3 - 5.5 μ m Physiology: Ferments glucose, lactose, sucrose; does not ferment raffinose; assimilates glycerol, mannitol, asporagine.	Can

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y60	MR _{ij} Fruits	Culture: bordeaux-red pigment formed on medium with iron; the pigment was absent on YM agar. Morphology: Cells 5-8 x 3-5 μ m Physiology: same as in Y6.	<i>Candida pulcherrima</i>
14	MR _{ij} Fruits, Tonto	Culture: pellicle; sediment formed; developed an aromatic odour; streak shiny, smooth, cream Morphology: pseudomycelium; cells ovoid, ellipsoid to elongate; 4-5 x 6-10 μ m. See Figure 5 Physiology: KNO ₃ positive; acid produced assimilation and fermentation slow.	<i>Dekkera bruxellensis</i>
17	MR _{ij} Fruits, Tonto	Culture: sediment and pellicle with a ring; streak butyrous, friable, cream, raised; after 4 weeks brownish-cream Morphology: cell shape and size varied; round, ovate, elongated, often pseudomycelium with multipolar budding; 4-14 x 3-7 μ m See Figure 2. Physiology: fermented glucose, galactose, and maltose very well; raffinose $\frac{1}{2}$, was weakly fermented, growth in KNO ₃ was negative the species grew weakly in vitamin-free broth; it growth on 50% (w/w) glucose-yeast extract was reasonable; there was little growth on 60% (w/w) glucose-yeast extract.	<i>Saccharomyces cerevisiae</i>

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y68	MR _{ij} Fruits Tonto	Culture: Cream Morphology: bipolar budding; cells ovate 4-9 x 2-5 μ m Physiology: Ferments glucose, galactose, cellobiose; assimilates glucose, salicin, and maltose, cellobiose, mannose is not fermented.	Han
Y20	MR _{ij} Fruits	Culture: same as in Y68 Morphology: budding, many clusters, cells ovate Physiology: Ferments glucose, sucrose, assimilates glucose, cellobiose, glucosamine, salicin, sucrose.	Han
Y21	MR _{ij} Fruits Tonto	Culture: Cream, pellicle formed Morphology: budding, multipolar cells elongate, glubose, 6-8 x 5-6 μ m. Physiology: ferments glucose, galactose, maltose, sucrose; assimilates glucose, lactic acid, glucoside, raffinose. Grew well at 37°C.	SaY21
Y12	MR _{ij} Tonto	Culture: Cream; sediment formed Morphology: Cells ellipsoid, multilateral budding 5-12 x 5-10 μ m Physiology: Ferments glucose weakly; ferments well maltose, cellobiose, sucrose, raffinose melibiose, assimilates glucose, galactose, sucrose, maltose, melibiose, trehalose, raffinose, melezitose, ethanol, glycerol, mannitol, lactic acid; grows well at 37°C.	SaY12 or S. <i>carlsbergensis</i>

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y26	MR _{ij} Fruits Tonto	Culture: White colony Morphology: Cells cylindrical to rectangular, ovoid or glubose, by fission Physiology: Ferments glucose, galactose, assimilates glucose, glucosamine, glucoside, raffinose, salicin, starch, weakly sarbose; grows well at 30°C, 37°C and 42°C.	ScY26
Y37	MR _{ij} Fruits	Culture: Yellow Morphology: amyloid compound produced Physiology: Does not ferment or is non-fermentative, assimilates glucose, galactose, glucosamine, sorbose.	CrY37
Y13	MR _{ij} , JM _{ij} , MJ Fruits, Millet, Sorghum and Tonto	Culture: Pellicle, then sediments streak white; friable Morphology: Cylindrical to rectangular, elongate, oval mycelium, 20-40 µm x 3-5 µm. See Fig. 1 Physiology: Fermented glucose, galactose, maltose, sucrose, raffinose, melibiose, growth on KNO ₃ , vitamin - free media was negative. Maximum growth temperature on malt extract was 43°C starch test positive.	<i>Schizosaccharomyces pombe</i>

Table 1 Cont...

STRAIN NO.	SOURCE	PARTICULARS	NAME
15	MR _{ij} Fruits & Tonto	Culture: Sediment formed, cream; butyrous; flat Morphology: Cells spheroidal, ovoid or cylindrical; 2.5 x 7 4-10 μm; cells occurred singles or in pairs; a few times formed chains. Fig. 3. Physiology: Glucose and sucrose were fermented, but galactose, maltose and lactose were not fermented. There was no growth in KNO₃.	<i>Saccharomyces chevalieri</i>
Y29	MR _{ij} Fruits	Culture: Matted, cream Morphology: Cells single; no formation of pseudomycellium or true mycelium Physiology: Amyloid compounds formed; fermented glucose, raffinose, assimilated glucose, erythritol, glucosamine, inositol, raffinose, salicin, sorbose, trehalose; grew at 37°C.	TY29
Y42	MRU _{ij} sorghum Muramba	Culture: Cream, wrinkled pellicle Morphology: Cells ovoid, more or less cylindrical, cells 5-10 x 3-5 μm Physiology: Fermented glucose only; assimilated glucose; salicin, maltose, cellobiose, glycerol; assimilated weakly succinate, citrate, asporagire, glutamic acid, glycine, lysine, glucosamine; grew well at 30°C, 37°C and 42°C.	CanY42

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y49	MRU _{ij} sorghum Muramba	Culture: Pinkish, dry pellicle Morphology: Cells spherical or ovoid, elongate; multipolar budding; pseudomycelium. Physiology: Ferments glucose, raffinose, assimilates glucose, ethanol, sucrose, maltose, lactose, raffinose, glycerol, trehalose, erythritol, mannitol, salicin; melezitose, KNO ₃ positive. Grow at 30°C.	HaY49
Y62	MRU _{ij} sorghum Muramba	Culture: Same as Y49 Morphology: Similar to Y49 Physiology: Fermented glucose only; assimilated glucose, salicin, maltose, cellobiose, but not trehalose, lactose, raffinose, or melezitose; did not grow at 30°C.	HaY62
Y67	MRU _{ij} Muramba	Culture: Cream Morphology: bipolar budding, cells ovate, 4-7 x 2-4 Physiology: Fermented glucose, galactose, lactose; lactose; assimilated glucose (weak), maltose, trehalose, raffinose, inulin (slowly), glycerol, salicin. Grew at 30°C but not above.	HanY67

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y30	MRU _{ij} Muramba	Culture: pellicle; cream Morphology: budding; cells round, ovate, large vacuoles; 4-14 x 3-7 μ m Physiology: Fermented glucose, sucrose (weak) cellobiose, trehalose, maltose, raffinose, Assimilated glucose, galactose, sucrose, trehalose, melibiose, inulin, mannitol, 30°C but not above.	SaY30
Y68	MRU _{ij} Muramba	Culture: Similar to Y30 Morphology: Similar to Y30 Physiology: Fermented glucose (weak) sucrose, cellobiose, trehalose, maltose, raffinose, melizitose, assimilated glucose, galactose, melibiose (weak), mannitol (weak), grew at 30°C but not above.	SaY68
Y56	MRU _{ij} Muramba	Culture: White, friable Morphology: Cells cylindircal, elongated, ovoid, or globose; limited mycelium, fission. Physiology: Fermented glucose, sucrose, cellobiose, % raffinose; assimilated glucose, sucrose, maltose; KNO ₃ positive grew at 30°C but not above produced amyloid compounds.	ScY56

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y57	MRU _{ij} sorghum	Culture: Yellow; matted, coherent Morphology: pseudomycelium Physiology: Fermented galactose, melizitose; Assimilated galactose, sorbose, melibiose, ethanol, glycerol, erithritol, salicin, late, succinate (weak), citrate (weak); grew at 30°C but not above.	CrY57
Y46	MRU _{ij} Muramba	Culture: Pellicle formed; cream Morphology: budding multilateral; cells spheroidal; globose. See Fig. 7 Physiology: Fermented glucose, sucrose, cellobiose, maltose, lactose, raffinose; Assimilated glucose, galactose, sorbose, maltose, cellobiose, trehalose, lactose, melibiose, starch, salicin, lactate; grew in vitamin-free medium; grew at 30°C but not above; nitrate negative.	DY46 <i>Debarryomyces</i> <i>hansenii</i>
Y48	MR _{ij} , MRU _{ij} Tonto, Muramba	Culture: Cream, butyrous; sediment with a ring, shiny, margin undulating. Morphology: Cells spheroidal, ovoid or elongate 3-7 x 4-18 µm; singly or in pairs or short chains or clusters. Fig. 1. Physiology: Fermented glucose, galactose (slow).	<i>S. pastorianus</i>

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
2	JT _{ii} : Amarwa Millet M _j : Amarawa	Culture: Yellow, wrinkled pellicle, matted Morphology: budding; pseudomycelium, producing clusters of blastospores cells ovoid to cylindrical. Physiology: Fermented glucose, sucrose; assimilates glucose, sorbose, sucrose, ethanol, glycerol, mannitol, lactate, succinate, glucoside, aspartic acid, glutamic acid; grew at 30°C and 37°C; grew at 50% glucose.	Ca2
11	JT _{ii} : Amarwa millet M _j : Amarwa Ainyeles	Culture: As in 2 Morphology: As in 2 Physiology: Fermented glucose, sucrose, assimilated galactose, sorbose (weak) sucrose, ethanol, glycerol, mannitol, lactate, succinate, glucoside, aspartic acid (weak) glutamic acid (weak).	Ca11
62	JT _{ii} : Amarwa millet M _j : Amarwa Ainyeles	Culture: Dry wrinkled pellicle; cream to redish Morphology: Cells spheroidal, round, ovate, elongate, forms pseudomycelium. Physiology: Vigorously fermented glucose, sucrose (weak) maltose; assimilated glucose, glycerol, and was KNO ₃ positive (utilised NO ₃). Grew at 30°C but not above.	Ha62

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
32	JT _{ij} Amarwa millet M _i Amarwa Ainyeles	Culture: same as in 62 Morphology: same as in 62 Physiology: Fermented glucose, galactose, sucrose maltose, Assimilated glucose, melibiose, (weak), glycerol, KNO ₃ (weak); Grew at 30°C but not above.	Ha32
30M	JT _{ij} Amarwa millet M _i Amarwa Ainyeles	Culture: Cream, sediment Morphology: Cells more or less ellipsoids 5-12 x 5-10 µm; budding Physiology: Fermented glucose, sucrose (weak), maltose, cellobiose, trehalose, Assimilated glucose, sorbose, melibiose (weak), mannitol (weak); grew at 30°C and 37°C but not higher.	Sa30M
30N	JT _{ij} Amarwa millet M _i Amarwa Ainyeles	Culture: As in 30M Morphology: As in 30M Physiology: Fermented glucose, sorbose, sucrose (weak), trehalose (slow); Assimilated glucose, galactose, sorbose (weak), sucrose, trehalose (slow), raffinose (weak) ethanol, glycerol, citrate, lactate. Grew at 30 but not above.	Sa30N

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
30P	JT _{ij} Amarwa millet M: Amarwa Ainyeles	Culture: Similar to 30M Morphology: Similar to 30M Physiology: Fermented glucose, sorbose, sucrose (slow), cellobiose, trehalose, Assimilated glucose, sucrose, maltose, trehalose, raffinose.	Sa30P
44M	JT _{ij} Amarwa millet M: Amarwa Ainyeles	Culture: White pellicle, then sediment Morphology: Cells cylindrical or rectangular, ovoid, elongated to glubose, fission 5-40 x 4-5 μ m Physiology: Fermented glucose, sucrose, maltose, Assimilated glucose, galactose, raffinose.	Sc44M
44N	JT _{ij} Amarwa millet M: Amarwa Ainyeles	Culture: Same as in 44M Morphology: Same as in 44 M Physiology: Fermented glucose, maltose, Assimilated glucose, galactose, sucrose, melibiose, raffinose, glucose, maltose.	Sc44N
44P	JT _{ij} Amarwa	Culture: As in 44M Morphology: As in 44M Physiology: Fermented glucose, sucrose, maltose; raffinose; Grew at 30°C.	Sc 44P

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
10	JT _{ij} Amarwa MR _{ij} Tonto	Culture: Sediment formed; streak butyrous; friable, cream, flat, entire margin, esterical aroma Morphology: budding, pseudomycelium branched formed blastospores in clusters Physiology: Fermented glucose (slow), galactose (weak), KNO ₃ negative.	<i>Lodderomyces elongisporus</i>
44	JT _{ij} Amarwa Millet M _i Amarwa Aingeles MR _{ij} Tonto Fruits	Culture: Sediment, cream Morphology: Cells ellipsoidal to ovoid, 6-8 x 56 µm Physiology: Fermented strongly glucose raffinose completely galactose, sucrose: Assimilated glucose, lactate, glucoside, raffinose completely, maltose, KNO ₃ negative. It differed from <i>S. cerevisiae</i> by fermenting raffinose completely.	<i>Sac. ellipsoides</i>
21	MR _{ij} Fruits Tonto	Culture: Sediment and a ring, cream, smooth shiny, margin entire, streak butyrous, Morphology: Spheroidal, Ovoidal, 3-7 x 3-9 µm, formed rudimentary pseudomycelium figure 4 Physiology: Vigorous fermentation of glucose, sucrose, raffinose (week); no growth in maltose	<i>Sa. capensis</i>

Table 1 Cont.

STRAIN NO.	SOURCE	PARTICULARS	NAME
50	MR _{ij} Tonto	Culture: Yellow, Morphology: Cells rods, ovoid, multi-polar budding Physiology: Fermentation nil; Assimilation glucose (weak) galactose, sorbose, lactose, glycerol, lactate, succinate, produced amyloid compounds	Cry
Y60	MR _{ij} Tonto	Culture: Dull, dry pellide, cream Morphology: Cells, round, short, oval, multipolar budding Physiology: Fermented glucose (weak), raffinose, mellibiose (weak), Assimilated glucose sorbose, salicin, melibiose, cellobiose, raffinose (weak), starch (weak) erythritol, lactate, aspartate, asparagine, glutamate, glycine. lysize; grew at 30°C and 37°C but not higher; grew on 50% (W/W) glucose (delayed).	DY 60

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y 25	MR _{ij} Tonto	Culture: Cream, sediment, pellicle not formed Morphology: pseudomycelium poor; bipolar budding bipolar, cells ellipsoids, ovoid, globose, 6 - 8 x 5 - 6 μ m Physiology: Fermented glucose, cellobiose, sucrose, raffinose (2/3), melibiose, Assimilated glucose, galactose, sucrose, maltose, melibiose, trehalose, raffinose (weak), melezitose, ethanol, glycerol, lactate (weak)	Sac Y 25
Y10	MR _{ij} Fruits	Culture: As in Y60 Morphology: As in Y60 Physiology: Fermented glucose, maltose (weak, delayed), sucrose, raffinose, melibiose (weak); Assimilated glucose, galactose, sorbose, sucrose, maltose (weak, delayed), raffinose, melezitose, inulin, glycerol, erythritol, mannitol, lactate, succinate, citrate, KNO ₃ positive, Aspartate, asparagine, glutamate, erythritol, glucoside; grew well at 30°C, 37°C and 42°C.	DY10

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y32	MR _{ij} Fruits	Culture: As in Y25 Morphology: As in Y25 Physiology: Fermented glucose, galactose, maltose, cellobiose, sucrose, raffinose, melibiose; Assimilated glucose, galactose, sucrose, maltose, melibiose, trehalose, raffinose, melitose, glycerol, lactate (weak), grew at 30°C, 37°C, 42°C.	SaY32
Y56A	MR _{ij} Fruits	Culture: White colony, friable; Morphology: Cells cylindrical/rectangular, elongate, ovate, or glubose; mycelium; fission Physiology: Fermented glucose, maltose, sucrose, raffinose, melibiose; Assimilated glucose, galactose, sucrose, maltose, raffinose; grew only up to 30°C; produced amyloid compounds.	ScY56A
Y57A	MR _{ij} Muramba	Culture: Light cream, matted Morphology: true mycelium; cells round, ovoid; multipolar budding. Physiology: Non-fermentative; Assimilates galactose, glucoside (delayed), lactate; produced amyloid compounds.	CrY57A

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y1	MR _{ij} ; JM _{ij} millet, Fruits Tonto, Kwete Amarwa	Culture: Glistening; pellicle Morphology: budding bipolar Physiology: Fermented glucose, raffinose, sucrose; Assimilated erythritol (weak), ethanol, glycerol, maltose, glucoside, raffinose (weak) succinate (weak), sucrose (weak, delayed); grew at 30°C, 37°C.	Y1 (SaY1)
Y2	MR _{ij} Fruits Tonto MRU _{ij} Muramba	Culture: Cream, pellicle, thin Morphology: Cells ovoid, elongate, ellipsoid Physiology: Fermented glucose, maltose (weak) ethanol (weak), glycerol (weak), lactose (weak), raffinose (weak), melibiose (weak), succinate (weak), sucrose (weak, delayed); grew at 30°C.	Y2 (SacY2)
Y4	MR _{ij} Tonto Fruits	Culture: Cream, smooth Morphology: Cells elongate Physiology: Ascospores produced, ovoid, glucose, cellobiose, galactose, maltose (weak) sucrose; Assimilated ethanol, galactose (weak, delayed), glycerol (weak), lactose (weak), raffinose, melibiose, salicin, succinate, sucrose, trehalose.	Y4 (HaY4)

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y5	MR _{ij} Tonto Fruits	Culture: Cream, sediment with a ring Morphology: Cells ovoid, elongate; spheroidal; Physiology: Fermented glucose, maltose, 1/4 raffinose (weak), sucrose; Assimilated ethanol (weak), galactose (weak), glycerol, inositol, lactose (weak), maltose, glucoside, raffinose (weak, delayed), sucrose (weak), grew at 30°C and 37°C, but not above.	Y5 (SacY5)
Y7	MR _{ij} Fruits	Culture: White; friable Morphology: Cells cylindrical, elongated, ovoid 4-40 x 3-6 µm. Physiology: Glucose, galactose (delayed, weak); maltose, sucrose (weak), assimilated ethanol (weak), galactose (weak), maltose, glucoside, raffinose, melibiose (weak) sorbose, sucrose (weak), grew at 30°C, 37°C, 42°C.	Y7 (ScY7)
Y8	MR _{ij} Tonto	Culture: Thin, folded pellicle; Morphology: Cells ovoid, spheroidal, budding Physiology: Fermented glucose, sucrose, assimilated erythritol, ethanol (weak), galactose, lactose (weak) maltose, raffinose (weak), melibiose (weak), sorbose, trehalose.	Y8

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y9	MR _{ij} Fruits Tonto	Culture: Cream, Smooth Morphology: Cells ovoid, ellipsoidal, clusters Physiology: Fermented glucose, galactose, raffinose, sucrose, Assimilated galactose, glucoside, lactose (weak), maltose (weak), raffinose, melibiose (weak), sorbose (weak), sucrose (weak), produced aromatic odour.	Y9 (HaY9)
Y14	MR _{ij} Fruits Tonto	Culture: Sediment with a ring Morphology: Cells budding, bipolar, cells elongate Physiology: Fermented glucose, galactose (weak, delayed), sucrose, ethanol (weak), glycerol (weak), inositol (weak), lactose (weak), maltose, raffinose (weak), melibiose (weak), sorbose, succinate (weak), sucrose (weak).	Y14
Y22	MR _{ij} Fruits Tonto	Culture: Similar to Y14 Morphology: Similar to Y14 Physiology: Produced ascospores, ovoid, Fermented glucose, raffinose, sucrose Assimilated ethanol (weak), lactose (weak), melibiose, salicin (weak), sorbose, succinate (weak), sucrose (weak), grew at 30°C, 37°C.	Y22

Table 1 Cont..

STRAIN NO.	SOURCE	PARTICULARS	NAME
Y28 (CrY28)	MR _{ij} Tonto	Culture: Matted, margine entire; a pellicle is formed; cream Morphology: Cells round, ovoid, multipolar budding. Physiology: Ability to ferment was either nil Cellobiose, glactose or very weak. Assimilated ethanol (weak), galactose, glycerol (weak), lactose (weak), maltose, raffinose, melibiose (weak), sorbose (weak), produced amyloid compound.	Y28
Y38	MR _{ij} Fruits, Tonto	Culture: Cream, matted Morphology: Mycelium produced, cells spheroidal Physiology: Fermented glucose, galactose, only i.e. a poor fermenter. Assimilated galactose, lactose, meltose, glucoside, raffinose, salicin, sorbose, grew at 30°C, 37°C and 42°C. produced emyloid compounds.	Y38 (CrY38)

KEY: Can = *Candida*, Han = *Hanseniaspora*; Sa = *Saccharomyces*; Sc = *Schizosaccharomyces*;
 Cr = *Cryptococcus*; T = *Torulopsis*; Ha = *Hansenula*; D = *Debaryomyces*.

Codes for MR_{ij}, MRU_{ij} and JT_{ij}/M_j see Appendix I.

Fig. 1 *Schizosaccharomyces pombe* after 96 hours on YMA

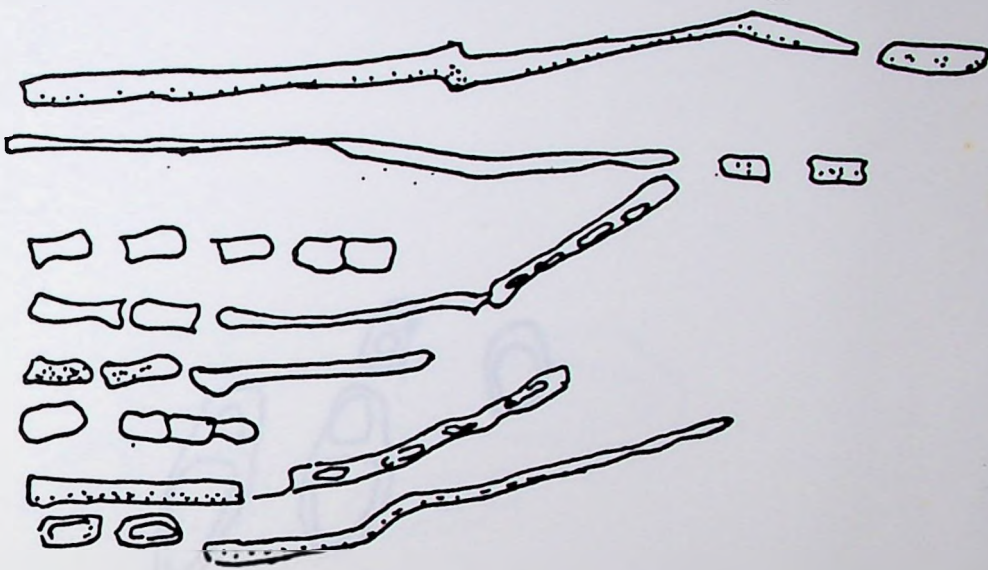


Fig. 2 *Saccharomyce cerevisiae* after 72 hours on
YMA



2

Fig. 3 *Saccharomyces chevalieri* after 72 hours on
YMA

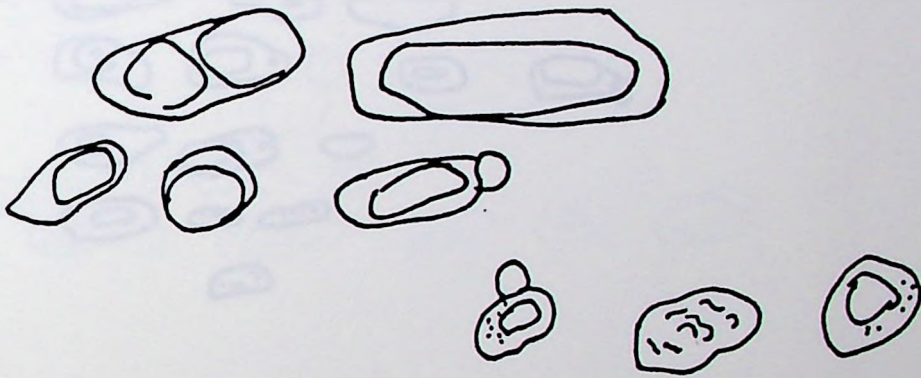


Fig. 4 *Saccharomyces capensis* after 48 hours on YMA



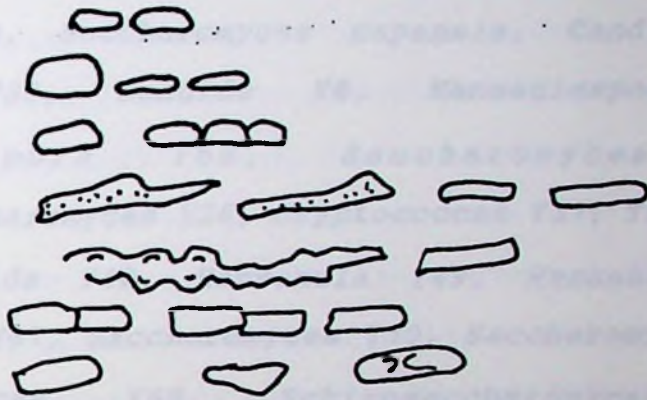
Fig. 5 *Dekkera bruxellensis* after 96 hours on YMA



Fig. 6 *Lodderomyces elongisporus* after 72 hours on
YMA



Fig. 7 *Debaryomyces hansenii* after 72 hours on YMA



The results of the total of 79 tests led to characterisation of 56 yeasts strains to genus and/or species. The species were: *Candida pulcherrima*, *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Schizosaccharomyces pombe*, *Saccharomyces chevalieri*, *Saccheromyces pastorianus*, *Lodderomyces elongisporus*, *Saccharomyces ellipsoides*, *Saccharomyces capensis*, *Candida* Y50, *Candida* Y33, *Candida* Y6, *Hanseniaspora* Y20, *Hanseniaspora* Y68, *Saccharomyces* Y21, *Schizosaccharomyces* Y26, *Cryptococcus* Y37, *Torulopsis* Y29, *Candida* Y42, *Hansenula* Y49, *Hansenula* Y62, *Hansenula* Y67, *Saccharomyces* Y30, *Saccharomyces* Y40, *Saccharomyces* Y68, *Schizosaccharomyces* Y56, *Cryptococcus* Y57, *Debaryomyces* Y46, *Candida* 2, *Candida* 11, *Hansenula* 62, *Hansenula* 32, *Saccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schizosaccharomyces* 44M, *Schizosaccheromyces* 44N, *Schizosaccheromyces* 44P, *Cryptococcus* Y50, *Debaryomyces* Y60, *Saccharomyces* Y25, *Debaryomyces* Y10, *Saccharomyces* Y32, *Schizosaccharomyces* Y56A, *Cryptococcus* Y57A, Y1 (*Saccharomyces* Y1), Y2 (*Saccharomyces* Y2), Y4 (*Hansenula*), Y5 (*Saccharomyces* Y5), Y7 (*Schizosaccharomyces* Y7), Y9 (*Hansenula* Y9), Y28 (*Cryptococcus* Y28), Y38 (*Cryptococcus* Y38). The following were characterised but not confirmed. Y8, Y14, Y22.

Doubtful cases arose as follows: Y1, Y2, Y5 were early vigorous fermenters of glucose but Y2 did not ferment raffinose, Y5 fermented the sugar weakly. In general Y1 reacted weakly in assimilative media, unlike *Saccharomyces*, generally.

The strain Y4 vigorously fermented D-glucose, collibiose, melibiose and assimilation salicin together with formation of ascospores. These characteristics pointed to *Hansenula*. However, Y4 did not utilise KNO_3 , Y9 did not ferment cellibiose and did not form ascospores, although the rest of the characteristics were similar to those of *Hansenula*. Y7 had similar characteristics to those of *Schizosaccharomyces* except that it was generally weak in reaction to the nutrients. Y28 and Y38 had many characteristics that pointed to *Cryptococcus* except that they fermented glucose. *Cryptococcus* is generally a non-fermenter. The two were generally poor fermenters though.

The unconfirmed Y8, Y14, Y22 could not match well with the available characteristics according to Lodder et al and van der Walt et al (1984).

Table VI shows yeast strains of generally unconfirmed or doubtful identity isolated from fruits, beverages and grains. Twelve isolates were characterised as follows: From fruits in Masaka/Rakai (Table I), Y1 (*Saccharomyces* Y1), Y2 (*Saccharomyces* Y2), Y4 (*Hansenula* Y4), Y5 (*Saccharomyces* Y5), Y7

(*Schizosaccharomyces* Y7), Y8, Y9 (*Hansenula* Y9), Y14, Y22, Y28 (*Cryptococcus*), Y38 (*Cryptococcus* 38). From tonto: Y1 (*Saccharomyces* Y1), Y2 (*Saccharomyces* Y2), Y4 (*Hansenula* Y4), Y5 (*Saccharomyces* Y5), Y8, Y9 (*Hansenula* Y9), Y14, Y22, Y28 (*Cryptococcus* Y28). From Muramba in Mbarara/Rukungiri: Y1 (*Saccharomyces* Y1), Y2 (*Saccharomyces* Y2).

3.2 Yeast biodiversity and density

Table V shows characteristics distinguishing yeast strains from Masaka/Rakai. This is a region where bananas are grown extensively and intensively and nearly every homestead indulges in tonto brewing. So it is the home of bananas and tonto. Ten yeast strains were isolated from fruits, largely banana fruit. The strains included: *Candida* Y50, *Cryptococcus* Y37, *Candida pulcherrima* (Y60), *Dekkera bruxelensis*, *Saccharomyces cerevisiae* (17), *Hansenula* Y68, *Hansenula* Y20, *Saccharomyces* Y21, *Schizosaccharomyces* Y26, *Schizosaccharomyces bombe*, *Saccharomyces chevalieri*, *Torulopsis* Y29.

Tables II, III and IV summarise the biodiversity and population density of yeast strains isolated from fruits and beverages in Masaka/Rakai (Table II), Mbarara/Rukungiri (Table III), fruits, grains and beverages in Jinja/Tororo/Mbale (Table IV). The yeast strain density differed and diversity varied remarkably when Central and South/West regions are

Table II Yeasts Associated with fruits, beverage and grains in Masaka/Rakai

	B	P	M	PA	PW	TO	K
<u>Fermentative Yeasts</u> (Totals in %)							
Candida Y50	74.9	3.6	11.2	2.7	2.2	4	13
Candida Y33	63.6	2.2	12	4	8.7	6.4	27
Candida Y6	48	5.2	24	6.0	5.3	7.5	38
Candida Y60	70.7	1.8	14	3.7	3.7	4.9	12
Hansenula 14	69.8	7.4	8.6	2.5	4.3	5.6	18
Hansenula Y17	72.8	5.2	12	3.5	3.5	2.9	0
Hanseniaspola Y68	59	2.6	21	58.8	4.6	3.9	26
Hanseniaspora Y20	68	2.8	19.9	2.2	3.6	3.3	39
Saccharomyces Y21	62.5	5.7	15.3	7.3	1.1	4.5	34
Saccharomyces Y25	67	12	9	6	1.5	3	15
Saccharomyces Y12	70.5	1	19.5	2	4	1.5	15
Schizosaccharomyces Y26	71.7	3	13.6	5.5	3.5	1	15
<u>Non-fermentative Yeasts</u>							
Cryptococcus Y11	36.9	12.8	19.8	8.16	12.82	4.66	13
Cryptococcus Y37	23.4	11.2	12.1	11.2	15.1	13.2	14
Debaryomyces Y13	48.8	7	16.8	4.6	8.4	8.4	6
Rhodotorula Y15	34	14.9	35.5	9.2	3.5	2.1	1
Rhodotorula Y24	48	15.4	10.3	14	11.3	0	1
Terulopsis Y29	63.3	16.3	10.2	5.4	1.8	1.8	12

Key: B = Banana P = Pineapple
M = Mango PA = Passion Fruit
PA = Passion Fruit PW = Pawpaw
TO = Tonto KW = Kwete

TABLE III Yeasts associated with fruits, beverages and
grains in Mbarara/Rukungiri

Fermentative Yeasts
(totals in %)

	B	P	M	PA	PW	TO	M
Candida Y42	60.2	7.3	22.2	2.1	2.1	2.1	38
Candida Y50	50.4	3.1	19.4	13.2	6.25	4	23
Hansenura Y49	64.6	9/5	12.2	2.1	2.1	1.6	79
Hansenura Y62	34.6	6.1	49.2	3.4	5	1.1	1
Hanseniospora Y67	56	5.8	18.7	13.8	4.9	3.1	22
Saccharomyces Y30	64	8.2	11.4	4.4	2.5	7.16	2
Saccharomyces Y68	59.2	4.9	19.7	7/7	3/7	3.5	14
Schizosaccharomyces Y56	57.5	11.9	22.8	2.7	1	2.7	14

Non-fermentative yeasts

Cryptococcus Y57	33.5	7	45.5	2.00	2.00	8	2
Debaryomyces Y46	29.8	22.4	29.1	4.2	6.9	7.67	0
Debraryomyces Y48	58.6	2.7	20.7	1	4.5	9.9	27

M = Muramba

TABLE IV Yeasts associated with fruits, beverages and grains in Jinja/Tororo/Mbale

	B	P	M	PA	AK	AI	AM	SO	MI	MAI
Candida 2	0	0	0	0	0	5	70	3	10	12
Candida 11	0	0	0	0	0	4	81	2	8	5
Hansenura 62	2	0	0	0	1	2	80	7	6	4
Hansenura 32	1	0	0	0	0	4	64	5	12	15
Saccharomyces 30M	21	3	0	0	0	0	32	14	10	20
Saccharomyces 30N	24	0	0	0	0	0	30	12	15	19
Saccharomyces 30P	18	0	0	0	0	0	29	13	17	28
Schizosaccharomyces 44M	0	0	0	0	0	0	73	6	7	6
Schizosaccharomyces 44N	0	0	0	0	0	0	79	2	1	9
Schizosaccharomyces 44P	0	0	0	0	0	0	88	2	1	9
Schizosaccharomyces 44T	0	0	0	0	1	5	76	4	6	8
<u>Non-fermentative yeasts</u>										
Cryptococcus 16	24	24	31	8	0	2	1	4	1	5
Debaryomyces 14	37	6	18	4	1	5	15	0	5	9

Key: B = Banana (*Musa* spp) AI = Ainyeles
P = Pineapple (*Anans* sp) AM = Amarwa/Ajon
M = Mango (*mangifera* sp) SO = Sorghum
PA = Passion Fruit (*Passflora* sp) MI = Millet
AK = Akiria nuajon MAI = Maize

compared with East/North East. The study was undertaken to investigate population biodiversity and population density of yeasts responsible for certain fermentation processes. The fermented products of bananas, sorghum and millet were *tonto*, *Muramba* and *amarwa* in Masaka/Rakai, Mbarara/Rukungiri and Jinja/Tororo/Mbale respectively. In Masaka/Rakai (Table II) bananas showed the highest number of isolates for both fermentative and non-fermentative yeast strains. In this region the mean count of the colony forming yeast units (c.f.u) on bananas was 74.4 cm^{-2} , in *tonto* the c.f.u were 5.07×10^7 . Biodiversity was 66.5% on bananas and in *tonto* was 4%. In Mbarara/Rukungiri the counts for bananas was 76.6 cm^{-2} and $5 \times 10^7 \text{ ml}^{-1}$ counts in *muramba*. Biodiversity was 55.8% on bananas and 3.2% *muramba*. In Jinja/Tororo/Mbale counts on bananas were 2.5 cm^{-2} and *amarwa* $1.5 \times 10^7 \text{ ml}^{-1}$; on millet 25 cm^{-2} . The biodiversity was 6% on bananas, 11.8% on millet and 63.4% from *amarwa*.

Table VII shows characteristics distinguishing yeast strains isolated from *tonto* in Masaka/Rakai. In all 12 strains were isolated. These were characterised as *Candida* Y33, *Candida* Y6, *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, *Hansenula* Y68, *Saccharomyces* Y21, *Saccharomyces bergensis*, *Saccharomyces* Y26, *Schizosaccharomyces pombe*, *Saccharomyces chevalieri*.

Table VIII shows characteristics distinguishing yeast strains isolated from fruits in Mbarara/Rukungiri. Seven strains were characterized. These were *Candida* Y42, *Cryptococcus* Y57, *Debaryomyces* Y10, *Hansenula* Y49, *Saccharomyces* Y56A, *Schizosaccharomyces* Y56.

Table V Characteristics distinguishing yeast strain isolated from

	CaY50	CrY37	DY13	HaY14	HanY20	KY10	PY21
<u>Ascospore</u>	-	-	+	-	-	-	-
<u>Ferments</u>							
D-glucose	+	-	W	+	+	+	-
Cellebiose	-	-	-	-	-	-	-
D-galactose	0	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-
Raffinose	+	-	+	-	-	-	-
Sucrose	+	-	-	-	+	-	-
<u>Assimilates</u>							
Glucose	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	-
Trehalose	-	-	+	+	-	-	-
Cellobiose	+	-	+	+	+	-	-
Citrate	-	-	-	-	-	-	-
Erythritol	-	-	+	-	-	-	0
Ethanol	-	-	-	-	0	0	-
D-galactose	-	+	-	-	-	-	-

Table V cont..

	CaY50	CrY37	DY13	HaY14	Har
D-glucoseamine	+	+	+	DH	
Glycerol	-	-	-	0	
Myo-inositol	-	-	-	0	
Inulin	-	-	0	-	
DL-lactate	-	0	0	-	
Citrate	-	-	+	-	
Lactose	-	-	0	-	
Maltose	-	-	-	-	
D-Mannitol	-	0	-	-	
2-Me-glucoside	D	D	-	-	
Raffinose	-	-	-	-	
Melibiose	0.WD	-	+	-	
Salicin	-	-	+	-	
Soluble starch	-	-	w	-	

- a = MR_{ij} . Where MR_{ij} = Masaka area: j = specific villages where samples were taken:
 MR_{2j} = Rakai area.
- b = Biovars are referred to by the following abbreviations.
- Ca = *Candida*, Cr = *Cryptococcus*; D = *Debaryomyce*
 Ha = *Hansenula*; Han = *Hanseniaspore*; K = *Kloeckera*;
 P = *Pichia*, R = *Rhodotorila*, Sa = *Saccharomyces*;
 Sch = *Schizosaccharomyces*; T = *Torulopsis*.
- c = $MR_i jY_i$, = unvarying specific characters are given in cases of failure to confirm with published descriptions (monographs)
- (-) = not applicable (for example, not variable in this species)
- + = positive response;
- O = negative response; w = weak response;
- D = delayed response: ND = test not preformed; the test is not necessary for speciesw of level testing.
- / = response varied in repeated tests.

Table VI Characteristics distinguishing yeast strains of unconfirmed identity isolated from fruits, beverages and grains whose characteristics did not conform with available conventional description of yeasts

	YI ^c	Y ²	Y ⁴	Y ⁵	Y ⁷	Y ⁸	Y ⁹	Y ¹⁴	Y ²²	Y ²³	Y ²⁸	Y ³⁸
Ascospores	0	0	+	0	0	0	0	0	+	0	0	0
Ferments												
D-glucose	+	+	+	+	+	+	+	+	+	+	+w	+
Cellebiose	0	0	+	0	0	0	0	0	0	0	0	0
D-galactose	0	0	+	0	+D/W	0	+	+W/D	0	+	0	+
Maltose	0	+w	+(w)	+	+	0	0	0	0	0	+w	0
Raffinose	+	0	0	+w	0	0	+	0	+	0	+w	0
Sucrose	+	0	+	+	+W	+	+	+	+	0	+w	0
<u>Assimilates</u>												
Cellobiose	0	0	0	0	0	0	0	0	0	0	0	0
Erythritol	+w	0	0	0	0	+	0	0	0	0	0	0
Ethanol	+	+w	+	+w	+w	+w	0	+w	+w	+w	+w	0
D-galactose	+W/D	0	+W/D	+w	+w	+	+	+w	0	+w	+	+

Table VI cont..

	YI ^c	Y ²	Y ⁴	Y ⁵	Y ⁷	Y ⁸	Y ⁹	Y ¹⁴	Y ²²	Y ²³	Y ²⁸	Y ³⁸
D-glucose amine	0	0	0	0	0	0	+	0	0	0	0	D
Glycerol	+w	+w	+w	+	0	0	0	+w	0	0	+w	0
Myo-inositol	0	0	0	+	0	0	0	+w	D	0	0	0
Inulin	0	0	0	0	0	0	0	0	0	0	0	0
DL-lactate	0	0	0	0	0	0	0	0	0	0	0	0
Lactose	0	+w	D	+w	0	+w	+w	+w	+w	+w	+w	+
Maltose	+	0	+	+	+	+	+w	+	+w	+	+	+
D-Mannitol	0	0	0	0	0	0	0	0	0	0	0	0
2-Me-glucoside	+	0	0	+	+	0	0	0	0	0	0	+
Raffinose	+w	+w	+	+w	+	+w	+	+w	+w	+w	+	+
Melibiose	+	+w	+	+W/D	+w	+w	+w	+w	+	+	+w	0
Salicin	+w	0	+	0	0	0	0	0	w	0	0	0
Soluble starch												
L-sorbose	D	D	-	0	+	+	+	+	D	+w	+w	+
Succinate	+w	+w	+	0	0	-	0	+w	+w	+	+w	0
Sucrose	+W/D	+W/D	+	+w	+w	D	+w	+w	+w	+	+w	0

Table VI cont..

	YI ^c	Y ²	Y ⁴	Y ⁵	Y ⁷	Y ⁸	Y ⁹	Y ¹⁴	Y ²²	Y ²³	Y ²⁸	Y ³⁸
A-a-trehalose	0	0	+	+	0	+	0	0	0	0	0	0
Grows												
W/O Vitamins	0	0	0	0	0	0	0	0	0	0	0	0
W/O biotin	0	0	0	0	0	0	0	0	0	0	0	0
At 30°C	+	+	+	+	+	+	+	+	+	+	+	+
At 37°C	+	0	0	+	+	+	0	0	+	+	0	+
At 42°C	+	0	0	0	+	+	0	0	0	+	0	+
Produces amyclose	0	0	0	0	0	0	0	0	0	0	+	+

- a = MR_{ij} where MR_{ij} = Masaka area; j = specific villages where samples were taken; MR_{2j} = Rakai area.
- b = Bivars are referred to by the following abbreviations: Ca = Candida, Cr = Cryptococcus; D = Debaryomyces; Ha = Hansenula; Han = Hanseniaspore; K = Kloeckera; P = Pichia; R = Rhodotorula; Sa = Saccharomyces; Sch = Schizosaccharomyces; T = Torulopsis.
- c = $MR_{ij}Y_z$ = unvarying specific characters are given in cases of failure to confirm with published descriptions (monographs).

Table VII Characteristics distinguishing yeast strains isolated from Tonto in Masaka/Rakai

	CaY33	CaY6	Cry50	DY13	DY60	HaY17	HaY14	HanY68	SY26	Sc12	SaY21	SaY25
Ascospors	-	-	-	-	-	-	-	-	-	-	-	-
<u>Ferments</u>												
D-glucose	+	+	-	+W	+W	+	+	+	+	+(W)	+	+
D-galactose	0	0	-	-	-	-	-	+	-	-	-	-
Maltose	-	-	-	D	D	-	-	-	+	+	-	-
Cellebiose	-	-	-	-	-	+	-	+	-	+	+	+
Lactose	+	+	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	-	-	-	-	-	-	+	+	+W	+
Raffinose	+	0	-	+	+	+	+½	-	+	+	+	+½
Melibiose	-	-	-	+	+W	0	0	-	+	+	+	+
<u>Assimilates</u>												
D-glucose	+W	+W	+W	+	+	+	+	+	+	+	+	+
D-galactose	-	-	+	-	-	-	-	-	+	+	+	+
L-Sorbose	+D	-	+	+	+	-	-	-	-	-	-	-
A-methyl-D-glucose	-	-	-	-	-	-	+	+	-	-	-	-
Salicin	-	-	-	+	+	-	-	+	-	-	-	-
Sucrose	-	-	-	-	-	0	+	-	+	+	+	+
Maltose	-	-	-	-	-	0	-	+	+	+	+	+
Melibiose	-	-	-	+	+	-	-	-	-	+	+	+

Table VII Cont..

	CaY33	CaY6	Cry50	DY13	DY60	HaY17	HaY14	HanY68	ScY26	Sa12	SaY21	SaY25
Cellobiose	-	-	-	+	+	-	-	+	-	-	-	-
Trehalose	-	-	-	-	-	+	0	-	-	+	+	+
Lactose	-	-	+	-	0	-	-	-	-	-	-	-
Raffinose	-	-	-	-	+W	-	-	-	+	+	+	+W
Melezitose	-	-	-	-	-	-	-	-	-	+	+	+
Inulin	-	-	-	-	-	-	-	-	-	+	+	+
Starch	-	-	-	+W	+W	-	-	-	-	-	+S	-
Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	+	+	+	-	-	-	-	-	-	+	+	+
Erythritol	-	-	-	+	+	-	-	-	-	+	+	+
Mannitol	+	+	0	-	-	-	-	-	-	+	-	-
Lactate	-	-	+	+	+	-	-	-	-	+	+	+W
Succinate	-	-	+	-	-	-	-	-	-	-	-	-
Citrate	-	-	0	-	-	-	+	-	-	-	-	-
Nitrate	+	0	0	-	-	+	+	-	-	-	-	-
Nitrate	-	-	-	-	-	+	+	-	-	-	-	-
Aspartate	-	-	-	+	+	+	+	-	-	-	-	-
Asparagine	+	+	-	+	+	+	-	-	-	-	-	-

Table VII cont..

	CaY33	CaY6	Cry50	DY13	DY60	HaY17	HaY14	HanY68	ScY26	Sa12	SaY21	SaY25
Glutamate	-	-	-	+	+	+	+	-	-	-	-	-
Glycine	-	-	-	+	+	+	+	-	-	-	-	-
Lysine	-	-	-	+	+	+	+	-	-	-	-	-
Glucosemine	-	-	D	D	D	-	-	-	-	-	-	-
2-Me-glucoside	-	-	-	-	-	-	-	-	-	-	-	-
Grows									+(w)	-	+w	-
At 30°C	-	-	-	-	+	-	-	-	+			
At 37°C	-	-	0	0	+	-	-	-	+	+	-	+
At 42°C	-	-	-	-	-	-	-	-	+	-	-	-
With 50% Glucose	0	0	+ / D	+ / D	+ / D	0	-	-	0	-	-	-
Produces amyclose	-	-	+	-	-	-	-	-	+	-	-	-

Table VIII Characteristics distinguishing yeast strains
isolated from fruits in Mbarara/Rukungiri

	CaY42	CrY57	DY10	HaY49	HanY62	SaY56A	
ScY32							
Ascospores	-	-	-	-	-	-	-
<u>Ferments</u>							
D-glucose	+	-	+	+	+	+	+
D-galactose	-	-	-	-	-	+	-
Maltose	-	-	w/D	-	-	+	+
Cellebiose	-	-	-	-	-	+	-
Lactose	-	-	-	-	-	-	-
Sucrose	-	-	+	-	-	+	+
Raffinose	-	-	+	-	-	+	+
Melibiose	-	-	+w	-	-	+	+
<u>Assimilates</u>							
D-glucose	+	-	+	+	+	+	+
D-galactose	-	+	+	-	-	+	+
L-Sorbose	-	-	+	-	-	-	-
A-methyl-D-glucose	+w	+D	-	-	-	-	-
Salicin	+	-	-	+	+	-	-
Sucrose	-	-	+	+	-	+	+
Maltose	+	-	w/D	+	+	+	+
Melibiose	-	-	-	-	-	+	-
Cellobiose	+	-	-	+	+	-	-
Trehalose	-	-	-	+	-	+	-
Lactose	-	-	-	+	-	-	-
Raffinose	-	-	+	+	-	+	+
Melezitose	-	-	+	+	-	+	-
Inulin	-	-	+	-	-	-	-
Glycerol	+	-	+	+	-	+	-
Erythritol	-	-	+	+	-	-	-
Mannitol	-	+w	+	+	-	-	-

Table VIII Cont..

	CaY42	CrY57	DY10	HaY49	HanY62	SaY56A	
ScY32							
Lactate	-	+	+	-	-	+W	-
Succinate	+W	-	+	0	-	-	-
Citrate	+W	-	+	0	-	-	-
Nitrate	+	-	+	+	-	-	-
Nitrate	-	-	-	0	0	-	-
Aspartate	+	0	+	+	-	-	-
Asparagine	+	0	+	+	-	-	-
Glutamate	+	0	+	+	-	-	-
Glycine	+	0	0	0	-	-	-
Lysine	+	0	0	0	-	-	-
Glucosemine	+	-	-	-	-	-	-
Erythritol	-	-	+	+	+	-	-
2-Me-glucoside	D	-	+	+	-	-	-
Xylose	-	-	-	-	-	-	-
<u>Grows</u>							
At 30°C	+	-	+	+	-	+	+
At 37°C	+Q	-	+	-	-	+	-
At 42°C	+	-	+	-	-	+	-
With 50% Glucose	-	-	-	-	-	-	-
Produces amyclose	-	+	-	-	-	-	+

Table IX shows characteristics distinguishing yeast strains isolated from Muramba in Mbarara/Rukungiri.

The following yeast strains were characterised: *Candida* Y50, *Candida* Y42, *Hansenula* Y49, *Hansenula* Y62, *Hanseniaspora* Y67, *Saccharomyces* Y30, *Saccharomyces* Y68, *Schizosaccharomyces* Y56, *Cryptococcus* Y57A, *Debaryomyces* Y46.

Table X shows characteristics distinguishing yeast strains isolated from amarwa in Jinja/Tororo. Eleven yeast strains were characterised: *Candida* 2, *Candida* II, *Hansenula* 62, *Hansenula* 32, *Saccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schizosaccharomyces* 44M, *Schizosaccharomyces* 44N, *Schizosaccharomyces* 44P, *Lodderomyces elongisporus* (not shown in the table - see Table I), *Saccharomyces ellipsoides*.

Table XI: This Table shows characterisation of yeast strain isolated from millet from Jinja/Tororo. This region uses a lot of millet in the preparation of amarwa. The strains were: *Candida* 2, *Candida* II, *Hansenula* 62, *Hansenula* 32, *Saccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schizosaccharomyces* 44M, *Schizosaccharomyces* 44N, *Schizosaccharomyces* 44P, *Saccharomyces ellipsoides*.

Table IX Characteristics distinguishing yeast strains isolated from Muramba in Mbarara/Rukungiri

	CaY42	CaY50	CrY57A	DY46	DY48	HaY49	HaY62	HaY63	HanY67	SaY30	SaY68	ScY56
Ascospors	-	-	-	-	-	-	-	-	-	-	-	-
<u>Ferments</u>												
Glucose	+	+	-	+	+	+	+	+	+	+	+W	+
Galactose	0	0	+	-	-	0	+	0	+	0	0	-
Sucrose	+	+	-	+	+	0	+W	0	0	+W	+	+
Cellebiose	-	-	-	+	+W/D	0	0	+W	0	+	+	+
Lactose	+	+	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	-	-	-	0	0	0	0	+	+	0
Maltose	-	-	-	+	+W	0	+W	+	0	+	+	0
Lactose	-	-	-	+	+	0	0	0	+	-	-	-
Raffinose	+	+	-	+	+	+	+W	0	0	+	+	+W
Inulin	-	-	-	-	-	-	-	-	-	-	-	-
Melizitose	-	-	+	-	-	-	-	-	-	0	+	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-
<u>Assimilates</u>												
Glucose	+	+	-	+	+	+	+	0	+W	+	+	+
Galactose	-	-	+	+	+	-	-	-	-	+	+	-
Sorbose	-	-	+	+	+	-	-	-	-	-	-	-
Sucrose	-	-	-	-	+W	0	0	0	0	+	0	+
Maltose	-	-	-	+	+	0	0	0	+	0	0	+

	CaY42	CaY50	CrY57A	DY46	DY48	HaY49	Ha62	HaY63	HanY67	SaY30	SaY68	ScY56
Cellobiose	+	+w	-	+	+	-	-	-	-	-	-	-
Trehalose	-	-	-	+	+	0	+	0	+	+	0	0
Lactose	-	-	-	+	+w	-	-	-	-	-	-	-
Melibiose	-	-	+	+	+	0	+	+	0	+	+w	-
Raffinose	-	-	-	0	0	0	0	0	+	0	0	0
Inulin	-	-	-	+	+	0	0	0	-	-	-	-
Starch	-	-	-	0	0	+	+	0	+	+	0	-
Ethanol	+	+	+	0	0	+	+	0	0	0	0	-
Glycerol	+	+	+	0	0	0	0	+	+	0	0	-
Erythritol	-	-	+	0	0	+	+	0	0	+	+	-
Mannitol	-	-	-	0	0	+	0	0	0	+	+w	-
Salicin	-	-	+	+	+	+	+	0	+	0	0	-
Lactate	-	-	+	+	0	0	0	0	0	0	0	-
Succinate	-	-	+w	+	0	0	0	0	-	-	-	-
Citrate	+w	0	+w	0	0	0	0	0	-	-	-	-
Nitrate	+w	+	-	-	-	+	+	+	-	-	-	+w

Table X Characteristics distinguishing strains of yeasts isolated from Amarwa in Jinja/Tororo

	Ca2 ^d	Ca11	Ha62	Ha32	Sa30M	Sa30N	Sa30P	Sc44M	Sc44N	Sc44P	Sc44T
Ascospors	-	-	-	-	-	-	-	-	-	-	-
<u>Ferments</u>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	+	0	0	+W	-	-	-	-
Sorbose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+W	+	0	+W	0	-	-	-	-
Maltose	-	-	+	+	+	+	+	0	0	0	0
Cellebiose	-	-	-	-	+	+	0	0	+	0	+W
Trehalose	-	-	-	-	+	+	0	0	0	0	0
<u>Assimilates</u>											
Glucose	+	+	+	+	+	+	+W	+	+	+	+
Sorbose	+	+W	-	-	+	+	+	+W	+	0	0
Sucrose	+	+	0	0	+	0	0	-	-	-	-
Maltose	-	-	0	0	0	0	0	0	0	0	0
Galactose	-	-	-	-	0	0	0	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	0	0	+	0	0	0	0	0	0
Lactose	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	-	-	+W	+	+W	+W	-	-	-	-
Raffinose	-	-	-	-	-	0	0	0	0	0	0

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see Key to abbreviations

a = Biovars are referred to by the following abbreviations.

Ca = Candida

Ha = Hansunula

SC = Schizosaccharomyces

ND = Test not done

0 = Negative response

- = Not available on this species

+

W = Weak response

S = Slow growth

see Key to abbreviations

- a = Biovars are referred to by the following abbreviations.
- Ca = Candida
- Ha = Hansunula
- SC = Schizosaccharomyces
- ND = Test not done
- 0 = Negative response
- = Not available on this species
- +
- = Positive response
- W = Weak response
- S = Slow growth

Table XI Characteristics distinguishing strains of yeasts isolated from Millet in Jinja/Tororo

	Ca ²⁺	Ca11	Ha62	Ha32	Sa30M	Sa30N	Sa30P	Sc44M	Sc44N	Sc44P	Sc44T
Ascospors	-	-	-	-	-	-	-	-	-	-	-
Ferments											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	+	-	-	+	+	+	+	+
Sorbose	-	-	-	+	-	-	-	-	-	-	-
Sucrose	+	+	-	+	+W	+W	+S	+	-	+	+
Maltose	-	+	-	+W	-	-	-	+	-	-	-
Cellebiose	-	-	-	-	-	-	+	-	-	-	-
Trehalose	-	+	-	-	+S	+S	+	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-
Assimilates											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	+	-	+	-	+	-	+	+	-	+
Sorbose	-	-	-	-	-	+W	-	-	-	-	0
Sucrose	+	+	-	+	+S	+	+	-	+	-	-
Maltose	-	+	+	+	-	-	+	-	-	+	-
Cellobiose	-	-	+	+	-	-	-	-	-	-	-
Trehalose	+	-	-	+	+	+S	+	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-	+	-	-	-

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see Key to abbreviations

- a = Biovars are referred to by the following abbreviations.
- Ca = Candida
- Ha = Hansunula
- SC = Schizosaccharomyces
- 0 = Negative response
- = Not available on this species
- +
- = Positive response
- W = Weak response
- S = Slow growth

Table XII shows characteristics distinguishing yeast strains isolated from amarwa/ajon in Mbale. A lot of amarwa or ajon (from Atesot community here) is consumed daily in the characteristic circular grass hatched huts. The following were the yeast strains characterised: *Candida* 2, *Candida* II, *Hansenula* 62, *Hansenula* 32, *Saccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schisosaccharomyces* 44M, *Schisosaccharomyces* 44N, *Schisosaccharomyces* 44P, *Saccharomyces ellipsoides*.

Table XIII shows characterisation of yeast strain isolated from ainyeles from Mbale. Ainyeles is prepared from malted (germinated millet) which is sun-dried in the yard, on a clean area often smeared with cow-dung. The millet after drying is ground to flour. It is technically the "green malt" or yeast which is mixed with roasted millet malt during fermentation stage of the amarwa, The roasted malted millet flour is called akiria nuajon by Atesot. The following were the yeast strains: *Candida* 2, *Candida* II, *Hansenula* 62, *Hansenula* 32, *Saccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schisosaccharomyces* 44N, *Schisosaccharomyces* 44P, *Schisosaccharomyces* 44T.

Table XII

Characteristics distinguishing strains of yeasts isolated from Amarwa in Mbale

	Ca2 ^d	Ca11	Ha62	Ha32	Sa30M	Sa30N	Sa30P	Sc44M	Sc44N	Sc44P	Sc44T
Ascospors	-	-	-	-	-	-	-	-	-	-	-
<u>Ferments</u>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	-	-	-	-	-
Sorbose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	0	+	0	0	0	0	0
Cellebiose	-	-	-	-	0	0	0	-	-	-	-
Trehalose	-	-	-	-	0	0	0	-	-	-	-
<u>Assimilates</u>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sorbose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	-	-	0	0	0	0	+	0	0
Galactose	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	-	-	+	+	+W	+	+	+W	+	W
Cellobiose	-	-	-	-	0	0	-	-	-	-	-
Trehalose	-	-	0	0	0	+	+	-	-	-	-
Lactose	-	-	0	+	-	-	-	-	-	-	-
Melibiose	-	-	-	-	0	+	0	-	-	-	-
Raffinose	-	-	0	0	0	+	+	+	0	0	0

[illegible]

Table XII Cont..

[illegible]

see Key to abbreviations

- a = Biovars are referred to by the following abbreviations.
- Ca = Candida
- Ha = Hansunula
- SC = Schizosaccharomyces
- ND = Test not done
- 0 = Negative response
- = Not available on this species
- + = Positive response
- W = Weak response
- S = Slow growth

Table XIII Characteristics distinguishing strains of yeasts isolated from Ainyeles in Mbale

	Ca2 ^d	Ca11	Ha62	Ha32	Sa30M	Sa30N	Sa30P	Sc44M	Sc44N	Sc44P	Sc44T
Ascospores	-	-	-	-	-	-	-	-	-	-	-
<u>Ferments</u>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	-	-	-	-	-
Sorbose	-	-	-	+	-	+	+	-	-	-	-
Sucrose	+	+	-	+	0	0	0	+	-	+	+
Maltose	-	+	0	0	0	0	0	0	0	0	0
Cellebiose	-	-	-	-	0	0	0	0	0	+	+
Trehalse	-	-	-	-	+	+	0	+	+	+	+
<u>Assimilates</u>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sorbose	+	+	-	-	0	0	0	0	0	0	0
Sucrose	-	-	-	-	0	0	0	-	-	-	-
Galactose	-	-	-	0	0	0	-	-	-	-	-
Maltose	-	-	0	0	+W	0	0	0	0	0	0
Cellobiose	-	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	0	0	+	0	0	0	0	0	0
Lactose	-	-	-	+	+	+W	+	-	-	-	-
Melibiose	-	-	-	-	-	+	+W	+	-	-	-
						+	+W	0	0	0	0

[illegible]

see Key to abbreviations

- a = Biovars are referred to by the following abbreviations.
- Ca = Candida
- Ha = Hansunula
- SC = Schizosaccharomyces
- ND = Test not done
- 0 = Negative response
- = Not available on this species
- +
- W = Weak response
- S = Slow growth

CHAPTER FOUR

4.0 Analysis and Experiments

4.1 Numerical characterisation of yeasts isolated from fruits, grains and beverages

Introduction

Numerical taxonomy aims at a more objective system of classification. This started originally with adoption of the Adamson Principle that all properties (characteristics) for classification should be given equal weights. As many diagnostic characters as possible are used for numerical analysis, and these are formulated as yes (+) or no (-) alternatives. In conventional characterisation, a particular yeast is identified by matching the observed cultural, morphological and physiological characteristics with those recorded in standard monographs such as those of van der Walt *et al* (1984) for yeast previously described.

Normally the term Numerical Taxonomy is applied to systems of classification which are basically Adamsonian but which the degree of similarity of yeast strains is assessed in quantitative rather than merely qualitative terms (Campbell, 1974).

There are many advantages in having some numerical estimate of the degree of phenetic similarity or difference between a pair of yeasts of

which the most obvious is that it can provide a rational basis for deciding the levels of taxonomic rank.

In statistics, the search for relatively homogeneous groups is called cluster analysis (numerical taxonomy).

Methods

Yeast strains included: DeY46, *Saccharomyces pastorianus* (48), CaY42, CaY42, CaY50, HaY49, HaY62, HanY67, SaY30, SaY68, ScY56, CaY33, CaY6, *Dekkera bruxellensis* (14), *Saccheromyces cerevisiae* (17), HanY68, HanY20, SaY21, SaY25, SaY12, ScY26, Ca2, Ca11, Ha62, Ha32, Sa30M, Sa30N, Sa30P, Sc44M, *Saccheromyces ellipsoides* (44), Sc44T, Cry57.

This particular selection was made for three reasons. First, there is a selection which includes *Candida* an important yeast in biomass production for S.C.P. production; the second selection includes "top yeasts", the *S. cerevisiae*, and the 'bottom yeast' *S. ellipsoides* and the third includes the non-fermentative yeast, *Cryptococcus*.

Characteristics: 34 variables have been used for the analysis. These include 12 sugars and their derivative (salicin), 2 polysaccharides, 3 alcohols two organic acids, 7 amino acids, vitamins, 3 levels of temperature.

Method: organisms were grown on basal media as described for carbon, nitrogen and vitamin-free. The concentration for carbon compounds, nitrogen compounds and vitamins were as described under physiological characterisation described earlier. Cells for growth were on glucose peptone broth. Growth was initiated at a cell concentration obtained by serial dilution as described earlier. The cultures were grown in three litre sterile containers at 24°C and growth was assessed at regular intervals as follows:

(a) Growth was measured by direct method of dry weight as follows: centrifuged, washed yeast cells were dried to constant weight at 100°C in an oven. Dry weight was determined by weighing on an electronic balance.

(b) Recording: growth was recorded in mg.

Growth of 0 to 9.9 mg was considered insignificant and recorded as (-) and growth of 10.0 mg and above was considered significant and recorded (+).

Results : Characterisation of the yeasts

The results were recorded in Tables XIV-XX. Although these yeasts had been characterised before, there was need to repeat the characterisation because of long storage (over 12 months) which would imply changes in behaviour of the yeasts. The results,

however, do not differ significantly from those recorded earlier and so no more comments about them is made here, apart from the following:

Table XIV: The table shows characteristics for primary fermentation of yeast strains. 31 yeast strains were used, on 11 carbon sources.

Table XV shows results of assimilation of carbon compounds (hexoses). Both weight in mg and (+) or (-) weights are included.

Table XVI: This table continues with carbon assimilation but disaccharides were used and similar recording as in Table XV is shown.

Table XVII: Utilisation of carbon compounds by yeast strains isolated from fruits, grains and beverages. The table shows assimilation of trisaccherides and polysaccharides. The same recording as before was used.

Table XVIII: This table shows reaction of the 31 yeast strains to 3 alcohols, 3 organic acids. Recording of results was made as before.

Table XIX: The table shows growth of the 31 yeast strains to KNO_3 , NaNO_2 salts. All strains grow well on the control (complete) medium.

Table XX: The table shows growth response of the yeast strain in vitamin-free medium and at 30°C , 37°C and 42°C .

Results of Physiological Characteristics for Numerical Analysis

Table XIV Utilisation of Carbon Compounds by yeast strains isolated from fruits, grains and beverages: Fermentation

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Table XV Assimilative utilization of Carbon Hexoses (in mg) by yeast strains isolated from fruits, grain and beverages: x >10 mg is significant (+)

Strain No	D-glucose	D-galactose	L-sorbose	Salicin
DeY46	10.8 +	11.2 +	10.6 +	6.4 -
Sa48	10.2 +	6.8 -	14.1 +	13.8 +
CaY42	12.2 +	5.7 -	4.2 -	3.3 -
CaY50	11.7 +	2.5 -	5.3 -	6.7 -
HaY49	15.3 +	4.4 -	6.8 -	11.1 +
HaY62	12.0 +	3.1 -	8.7 -	5.6 -
HanY67	10.1 +	1.2 -	7.1 -	0.1 -
SaY68	13.4 +	6.6 -	6.5 -	6.6 -
ScY56	14.8 +	17.2 +	5.5 -	2.3 -
CaY33	9.9 -	2.2 -	10.9 +	2.1 -
CaY6	7.7 -	8.4 -	5.6 -	3.5 -
ScY56	12.8 +	5.5 -	7.4 -	4.2 -
Dekk 14	11.3 +	3.2 -	6.7 -	6.2 -
Sa17	14.9 +	3.4 -	9.9 -	4.1 -
HanY68	10.1 +	3.1 -	3.3 -	13.2 -
HanY20	13.1 +	2.2 -	8.7 -	8.1 -
SaY25	12.4 +	15.7 +	5.6 -	6.3 -
SaY12	10.6 +	16.8 +	3.4 -	6.6 -
ScY26	13.7 +	10.1 +	2.2 -	8.7 -
Ca2	17.4 +	8.8 -	17.9 +	6.6 -
Ca11	16.9 +	8.7 -	9.9 -	5.2 -
Han62	15.3 +	6.4 -	1.2 -	9.8 -
Ha32	10.2 +	6.9 -	8.9 -	3.7 -
Sa30M	12.4 +	5.4 -	10.3 +	1.1 -
Sa30N	11.7 +	6.3 -	4.7 -	9.8 -
Sa30P	9.9 -	5.5 -	5.6 -	9.7 -
Sa44	10.6 +	9.9 -	6.2 -	4.1 -
Sc44N	12.7 +	14.0 -	3.6 -	5.2 -
Sc44T	15.1 +	8.7 -	6.6 -	9.7 -
CrY57	8.6 -	10.2 -	1.1 -	8.7 -
Sa21	11.8 +	13.2 +	6.2 -	4.5 -

Table XVI Assimilative utilisation of carbon compounds by
yeast strains isolated from fruits, grains and
beverages

DISACCHARIDES

Strain No	Sucrose	Maltose	Melibiose	Cellebiose	Trehalose	Lactose
DeY46			10.1 +	12.4 +	6.3 -	6.7 -
Sa48	3.6 -	5.7 -	14.9 +	15.3 +	5.1 -	5.4 -
CaY42	5.5 -	4.5 -	6.1 -	14.8 +	6.4 -	4.3 -
CaY50	2.2 -	4.3 -	5.2 -	8.9 -	6.3 -	7.4 -
HaY49	10.2 +	2.2 -	2.6 -	10.6 +	10.3 +	10.6 +
HaY62	9.9 -	4.4 -	16.2 +	6.6 -	10.4 +	7.5 -
HanY67	8.8 -	6.5 -	4.9 -	5.6 -	14.2 +	8.3 -
SaY30	12.9 +	14.2 +	10.2 +	3.4 -	16.9 +	6.4 -
SaY68	6.9 -	5.5 -	3.9 -	4.7 -	8.9 -	6.5 -
ScY56	11.8 +	2.2 -	2.5 -	3.9 -	6.8 -	5.3 -
CaY33	5.7 -	7.3 -	4.4 -	2.5 -	5.7 -	4.2 -
CaY6	4.5 -	5.4 -	2.7 -	6.4 -	9.8 -	8.4 -
Dekk14	15.0 +	3.7 -	8.5 -	12.9 +	10.9 +	6.6 -
Sa17	5.9 -	4.2 -	6.2 -	8.8 -	10.9 +	5.5 -
HanY68	7.3 -	6.6 -	5.3 -	15.4 +	6.5 -	2.5 -
HanY20	12.1 +	5.4 -	6.2 -	14.3 +	3.8 -	1.6 -
SaY21	16.4 +	8.8 -	10.4 +	6.6 -	7.4 -	1.5 -
SaY25	14.3 +	9.9 -	16.3 +	8.3 -	12.1 +	2.3 -
SaY12	16.8 +	14.1 +	15.8 +	7.5 -	16.5 +	6.4 -
ScY26	10.6 +	10.5 +	7.6 -	7.8 -	5.8 -	5.4 -
Ca2	10.9 +	1.2 -	8.3 -	5.2 -	5.4 -	7.3 -
Ca11	14.8 +	7.6 -	9.5 -	4.6 -	5.8 -	6.2 -
Han62	0.9 -	6.8 -	9.5 -	9.7 -	3.7 -	5.1 -
Ha32	0.8 -	5.7 -	9.9 -	8.2 -	2.2 -	4.6 -
Sa30M	14.7 -	7.2 -	14.8 +	6.1	16.8 +	6.5 -
Sa30N	8.6 -	8.9 -	8.9 -	7.1 -	1.4 -	7.7 -
Sa30P	3.7 -	7.5 -	6.9 -	5.6 -	1.3 -	5.3 -
Sc44M	5.6 -	3.8 -	4.3 -	3.2 -	2.9 -	6.4 -
Sa44	6.4 -	1.2 -	3.5 -	3.9 -	7.9 -	7.5 -
Sc44T	6.8 -	6.2 -	7.6 -	9.8 -	9.4 -	6.6 -
CrY57	8.3 -	7.3 -	10.7 -	8.9 -	8.5 -	6.6 -

Table XVII Utilization of Carbon compounds by yeast strains isolated from fruits, grains and beverages

Trysaccharides and Polysaccharides

Strain No	Rifinose	Melezitose	Inulin	Starch
DeY46	11.8 +	13.6 +	0.3 -	12.4 +
Sa48	8.6 -	5.5 -	6.1 -	6.8 -
CaY42	8.9 -	4.3 -	5.3 -	4.1 -
CaY50	10.6 +	3.4 -	3.2 -	5.1 -
HaY49	16.6 +	14.6 +	2.3 -	6.5 -
HaY62	0.8 -	5.5 -	6.4 -	8.3 -
HanY67	14.2 +	8.6 -	0.9 -	6.6 -
SaY30	6.6 -	6.3 -	12.8 +	5.4 -
SaY68	5.7 -	10.9 +	3.9 -	4.2 -
ScY56	4.5 -	5.6 -	4.1 -	3.1 -
CaY33	3.4 -	4.4 -	5.2 -	2.3 -
CaY6	8.5 -	6.5 -	6.1 -	4.2 -
Dekk14	6.6 -	6.5 -	6.1 -	4.2 -
Sa17	7.3 -	3.6 -	8.2 -	8.1 -
HanY68	7.7 -	66.6 -	6.2 -	3.2 -
HanY20	6.6 -	7.4 -	5.1 -	5.2 -
SaY21	10.4 +	17.6 +	4.2 -	7.1 -
SaY25	8.9 -	16.4 +	3.6 -	6.2 -
SaY12	17.7 +	16.6 +	0.9 -	9.3 -
ScY26	10.9 +	0.4 -	6.2 -	0.9 -
Ca2	6.6 -	0.2 -	0.4 -	9.1 -
Ca11	5.4 -	0.6 -	0.1 -	2.2 -
Ha62	4.5 -	0.5 -	0.2 -	5.3 -
Ha32	3.3 -	0.4 -	0.3 -	4.1 -
Sa30M	2.7 -	0.3 -	0.1 -	5.1 -
Sa30N	8.8 -	0.2 -	0.1 -	6.1 -
Sa30P	9.9 -	0.6 -	0.2 -	0.3 -
Sa44	7.4 -	0.7 -	0.1 -	0.4 -
Sc44N	4.6 -	0.8 -	0.3 -	0.8 -
Sc44T	5.7 -	0.5 -	0.6 -	0.6 -
CrY57	6.9 -	10.3 +	0.4 -	0.1 -

Table XVIII Utilisation of Carbon compounds by yeast strains
isolated from fruits, grains and beverages
Alcohols and Acids

Strain No	Ethanol	Glycerol	Mannitol	Lactate	Succinate	Citralin
DeY46	14.1 +	12.3 +	11.0 +	14.2 +	12.7 +	1.9 -
Ba48	10.6 +	6.7 -	4.4 -	13.4 +	14.4 +	4.8 -
CaY42	10-9 +	10.6 +	5.7 -	3.2 -	10.0 +	6.9 -
CaY50	10.2 +	5.2 -	3.1 -	5.3 -	3.2 -	7.5 -
HaY49	16.2 +	4.6 -	11.2 -	3.2 -	2.4 -	6.3 -
HaY62	17.3 +	3.3 -	2.4 -	4.5 -	4.3 -	5.2 -
HanY67	3.6 -	10.8 +	6.6 -	2.4 -	6.2 -	3.8 -
BaY30	5.5 -	5.2	13.6 +	1.3 -	3.6 -	2.5 -
BaY68	4.2 -	4.4 -	8.9 -	4.5 -	2.1 -	4.2 -
ScY56	8.5 -	0.9 -	7.3 -	6.4 -	5.3 -	6.1 -
CaY33	6.4 -	13.4 +	13.7 +	2.6 -	4.2 -	4.3 -
CaY6	7.2 -	14.4 +	12.6 +	6.4 -	6.2 -	4.2 -
Dekk 14	5.3 -	3.2 -	4.3 -	5.4 -	6.6 -	10.1 +
Ba17	3.2 -	6.4 -	6.8 -	4.2 -	7.4 -	6.1 -
HanY68	2.8 -	8.6 -	3.3 -	3.2 -	8.5 -	8.2 -
HanY20		7.7 -	2.4 -	2.1 -	5.3 -	9.5 -
BaY21	10.3 +	10.5 +	9.9 -	10.6 +	4.4 -	7.6 -
BaY25	10.4 +	14.8 +	5.6 -	10.7 +	6.6 -	7.7 -
BaY12	4.6 -	12.6 +	10.9 +	13.5 +	7.1 -	5.2 -
ScY26	0.7 -	8.4 -	6.5 -	11.2 +	7.6 -	6.4 -
Ca2	10.3 +	10.6 +	14.7 +	15.1 +	10.3 +	16.1 +
Ca11	12.4 +	14.3 +	10.5 +	14.0 +	18.1 +	14.9 +
Ha62	5.3 -	12.4 +	7.6 -	4.3 -	3.2 -	7.2 -
Ha32	3.4 -	10.3 +	6.4 -	6.4 -	4.6 -	8.6 -
Ba30M	12.3 +	7.4 -	15.6 +	5.4 -	3.3 -	9.2 -
Ba30N	6.4 -	5.9 -	5.9 -	2.8 -	5.2 -	6.1 -
Ba30P	8.5 -	6.7 -	4.8 -	4.6 -	8.1 -	5.1 -
Sc44M	7.6 -	7.2 -	5.2 -	3.7 -	8.2 -	4.2 -
Ba44	6.1 -	6.8 -	6.6 -	2.2 -	7.9 -	5.9 -
Sc44T	4.2 -	8.5 -	6.3 -	1.9 -	2.6 -	3.3 -
CrY57	6.4 -	7.2 -	10.0 +	14.2 +	8.9 -	4.9 -

Table XIX Utilisation of Nitrogen Compounds by yeast strains isolated from fruits,
grains and beverages

Strain No	Control (complete)	Nitrate	Nitrite	Aspart	Arsp	Clut	Crtit	Citr.	Org.
DeY46	30.0 +	10.3 +	10.4 +	0.4 -	5.6 -	0.6 -	29.1 +	30.0 +	35.6 +
Sa48	34.0 +	8.4 -	10.7 +	0.3 -	6.4 -	11.0 +	11.4 +	18.6 +	20.6 +
CaY42	35.0 +	11.5 +	2.8 -	2.1 -	5.0 -	9.0 -	15.7 +	20.3 +	31.4 +
CaY50	38.0 +	16.7 +	3.6 -	10.3 +	10.6 +	6.0 -	29.8 +	25.6 +	33.0 +
HaY49	40.0 +	10.9 +	5.1 -	17.0 +	10.5 +	12.0 +	40.5 +	38.5 +	29.0 +
HaY62	19.0 +	16.3 +	7.1 -	11.0 +	10.3 +	6.9 -	32.1 +	37.7 +	20.0 +
HanY67	25.0 +	5.2 -	6.4 -	16.0 +	10.4 +	18.0 +	18.0 +	13.5 +	16.7 +
SaY30	30.4 +	4.1 -	5.2 -	0.2 -	4.1 -	7.6 -	32.3 +	17.6 +	20.0 +
SaY68	18.0 +	3.1 -	3.1 -	6.7 -	5.6 -	6.9 -	34.6 +	19.7 +	15.6 +
ScY56	18.7 +	10.2 +	2.6 -	6.6 -	3.7 -	10.1 -	30.4 +	30.0 +	37.1 +
CaY33	15.4 +	14.6 +	1.1 -	5.0 -	0.7 -	0.8 -	0.7 -	0.6 -	21.6 +
CaY6	40.0 +	2.1 -	1.3 -	0.1 -	0.8 -	6.0 -	28.1 +	23.0 +	35.6 +
Dekk14	36.4 +	10.6 +	5.1 -	0.7 -	0.6 -	4.7 -	18.0 +	17.6 +	30.6 +
Sa17	24.0 +	15.7 +	5.2 -	1.2 -	0.2 -	5.8 -	23.4 +	13.6 +	20.1 +
HanY68	26.0 +	16.8 +	3.3 -	10.0 +	0.3 -	8.4 -	10.1 +	11.1 +	22.6 +
HanY20	30.1 +	6.6 -	5.1 -	16.3 +	0.4 -	3.3 -	36.8 +	17.0 +	20.3 +
SaY21	38.0 +	6.4 -	6.1 -	17.0 +	0.1 -	0.0 -	25.6 +	17.6 +	34.6 +
SaY25	39.2 +	5.2 -	7.1 -	66.6 -	1.5 -	4.1 -	11.7 +	18.8 +	39.5 +

Table XIX Cont..

Strain No	Control (complete)	Nitrate	Nitrite	Aspart	Arsp	Clut	Crtit	Citr.	Org.
SaY12	34.1 +	8.3 -	6.2 -	6.2 -	25.0 +	10.1 +	10.3 +	24.0 +	25.6 +
ScY26	39.0 +	9.3 -	1.0 -	6.1 -	0.3 -	6.6 -	22.0 +	40.0 +	30.7 +
Ca2	46.0 +	7.1 -	8.2 -	2.3 -	0.4 -	3.9 -	23.4 +	17.8 +	33.3 +
Ca11	48.5 +	5.2 -	3.6 -	8.8 -	1.9 -	11.0 +	24.8 +	30.1 +	29.9 +
Ha62	28.5 +	16.6 +	2.1 -	6.1 -	0.7 -	12.0 +	28.8 +	27.0 +	34.5 +
Ha32	30.4 +	3.9 -	4.1 -	10.2 +	11.0 +	15.7 +	33.3 +	25.0 +	30.4 +
Sa30M	36.7 +	2.1 -	5.2 -	11.0 +	12.0 +	18.2 +	16.6 +	40.0 +	36.6 +
Sa30N	39.3 +	4.1 -	4.3 -	2.0 -	0.6 -	2.0 -	19.9 +	29.6 +	25.6 +
Sa30P	41.1 +	0.1 -	4.1 -	4.0 -	0.4 -	11.7 +	20.0 +	20.1 +	24.1 +
Sc44M	37.7 +	0.2 -	5.2 -	0.8 -	0.5 -	4.8 -	11.4 +	17.7 +	20.0 +
Sa44	35.6 +	0.1 -	6.1 -	0.7 -	0.6 -	3.7 -	20.0 +	16.0 +	20.0 +
Sc44T	30.4 +	0.1 -	7.1 -	6.0 -	0.3 -	4.6 -	29.5 +	16.1 +	27.4 +
CrY57	29.1 +	0.1 -	6.1 -	0.6 -	0.5 -	0.6 -	20.1 +	18.6 +	14.7 +

Table XX Growth: In Vitamin-free medium; on 50%
(w/w) glucose-yeast extract agar; at 30°C,
42°C by yeast strains isolated from fruits,
grains and beverages.

Strain No	V.f (w/o)	50% glucose	30°C	37°C	42°C
DeY46	6.3 -	6.4 -	10.6+	0.6 -	0.0 -
Sa48	12.4 +	3.1 -	12.8 +	3.1 -	0.2 -
CaY42	10.2 +	1.2 -	41.9 +	10.2 +	0.0 -
CaY50	10.8 +	1.1 -	40.6 +	10.6 +	0.0 -
HaY49	7.6 -	3.2 -	16.7 +	14.0 +	0.1 -
HaY62	16.7 +	2.0 -	17.8 +	16.0 +	0.1 -
HanY67	2.1 -	3.1 -	26.7 +	8.6 -	0.1 -
SaY30	4.2 -	2.3 -	20.4 +	6.7 -	0.3 -
SaY68	5.3 -	4.6 -	29.6 +	10.6 +	0.4 -
ScY56	3.6 -	1.1 -	30.4 +	5.6 -	0.5 -
CaY33	2.1 -	1.2 -	28.3 +	0.3 -	0.1 -
Dekk14	4.3 -	6.9 -	12.0 +	6.6 -	0.4 -
Sa17	2.1	7.7 -	10.1 +	7.8 -	0.0 -
HanY68	1.1 -	1.8 -	10.3 +	8.1 -	0.0 -
HanY20	2.1 -	7.7 -	10.6 +	0.9 -	0.0 -
SaY21	5.1 -	2.4 -	40.2 +	0.8 -	0.0 -
SaY25	3.3 -	6.6 -	38.4 +	0.6 -	0.0 -
SaY12	3.2 -	7.8 -	34.9 +	0.7 -	0.0 -
ScY26	6.1 -	2.1 -	20.7 +	9.9 -	0.0 -
Ca2	1.7 -	2.6 -	21.5 +	8.6 -	0.0 -
Ca11	2.3 -	4.9 -	23.6 +	5.7 -	0.0 -
Han62	6.7 -	3.1 -	40.7 +	9.9 -	0.0 -
Ha32	3.6 -	2.1 -	23.3 +	8.1 -	0.0 -
Sa40M	2.7 -	3.0 -	29.7 +	9.3 -	0.0 -
Sa30N	3.8 -	2.1	30.6 +	6.5 -	0.0 -
Sa30P	5.6 -	6.6 -	34.4 +	7.2 -	0.0 -
Sc44M	6.6 -	7.7 -	29.9 +	2.7 -	0.0 -
Sa44	3.7 -	6.2 -	28.7 +	9.9 -	0.0 -
Sc44T	4.4 -	6.0 -	20.1 +	8.9 -	0.0 -
CrY57	7.2 -	7.6 -	14.5 +	1.3 -	0.0 -

Numerical Analysis

Method:

Fermentation reaction results and growth results shown in Tables XIV to XX were analyzed by computer using procedure cluster SPSS/PC+ and techniques developed by Sneath & Sokal (1973).

A set of 70 characteristics formulated as (+) or (-) alternatives were used. The following procedure was followed: according to Hughes (1988)

- (a) Selection of yeast strains to be compared which were called operational Taxonomic Units or OTUs.
- (b) Observing these OTUs for the presence or absence (+,-) of quantity of a large set of characters. These were set in Tables XIV to XX.
- (c) Calculating distances or similarity coefficients using vectors of cosines.
- (d) Forming clusters.
- (e) Performing a cluster analysis.

The degree of relatedness between OTUs is a function of the number of similar characters in proportion to the total number of characters examined. The similarities between pair of strains is then expressed by a similarity coefficient S value, which is defined as:

$$S = \frac{a + b}{a+b+c+d} = \frac{a + d}{n} \dots\dots (i)$$

where a and d are the sums of the characters which are common to OTU A and OTU B (a both positive, d both negative). b is the sum of the characters in which A is positive and B is negative, and C is the sum of the characters in which A is negative and B is positive, giving the familiar 2 x 2 table.

		+	OTU B	-
	+	<u>a</u>		<u>b</u>
OTU A				
	-	<u>c</u>		<u>d</u>

After an OTU character table (matrix) has been compiled and all possible pairs of OTUs are compared and their similarities computed, there are three basic methods by which measures of similarity may be calculated.

These are:

1. Correlation coefficients;
2. Measures of taxonomic distances, coefficient, D.
3. Similarity coefficient.

Measure of distance was used in this study. Distance coefficients were calculated using a programme developed, based on SPSS/PC+, Statistical Guide for Similarity Coefficients. Distance measure was specified by cosine of vectors of variables:

$$\text{Distance (X, Y)} = \frac{\Sigma(X,Y)}{\Sigma(X^2) (Y^2)} \dots\dots(ii)$$

This expression is an extension of (i).

The results were recorded in Table XXI. After numerical estimate of the degree of similarity, in terms of distance coefficients of all possible pairs of OTUs the next step was to form groups or clusters which formed the basis of the final classification. The coefficients were clustered according to Everitt (1980), by agglomerative, hierarchical cluster analysis, using complete linkage. The results were recorded in Table XXI. The results were represented in horizontal and vertical icedcles to show degree of association of fusion by complete linkage. The results are shown in Figures 8 and 9. For each character two figures were calculated: the variance/cluster of character over all the yeast strains and the mean score for character minus overall mean score for character/overall standard deviation. A value of zero indicated homogeneity of a character within a cluster. This permitted identification of the test characters that were most distinctive for a given cluster. These values were recorded in Table XXII. Finally a dendrogram was drawn (Fig. 10) which resembles the diagrams of hierarchical classification, or dichotomous classification but differed in having quantitative estimates of similarities rather than qualitative criteria to determine its branching points.

Data Information

24 unweighed cases accepted

12 cases rejected because of missing value

Cosine measure used

1 Agglomeration method specified.

Agglomeration Schedule using Complete Linkage

Stage	Clusters cluster 1	Combined cluster 2	Coefficient	Stage Cluster Cluster 1	1st Appears Cluster 2	Next Stage
1	22	23				
2	1	2	.754652	0		
3	19	20	.639485	0	0	11
4	4	5	.557675	0	0	14
5	8	18	.513173	0	0	7
6	12	13	.445445	0	0	8
7	6	19	.405114	0	0	15
8	4	9	.385364	0	0	16
9	3	11	.372703	4	3	19
10	14	15	.330766	0	0	15
11	22	24	.324277	0	0	18
12	10	21	.305065	0	0	17
13	17	22	.249756	1	0	13
14	1	16	.225075	0	0	17
15	4	8	.222620	0	11	18
16	7	12	.066030	2	0	19
17	10	14	.031952	8	5	21
18	3	17	-.010638	0	6	20
19	1	6	-.101648	12	10	20
20	7	10	-.164051	9	13	21
21	3	4	-.164517	14	7	22
22	1	7	-.261571	16	17	22
23	1	3	-.467494	18	15	23
			-.558659	19	20	23
				22	21	0

Horizontal icicle plot using complete linkage

Number of Clusters



Table XXII Hierarchical Cluster Analysis : Stage II

Number of Valid Observations (Listwise) = 24.00

Variable	Mean	Std. Dev.	Minimum	Maximum	N Label
D-Glucose	12.25	2.35	7.7	17.4	31
D-galactose	7.53	4.42	1.2	17.2	31
L-sorbose	6.93	3.61	1.1	17.9	31
Salicin	6.35	3.33	0.1	13.8	31
Sucrose	8.79	4.50	0.8	16.8	31
Maltose	6.43	3.26	1.2	14.2	31
Melibiose	8.19	4.12	2.5	16.3	31
Cellebiose	7.84	3.97	0.1	15.4	31
Trehalose	7.95	4.26	1.3	16.9	31
Lactose	5.89	2.02	1.5	10.6	31
Raffinose	7.81	3.80	0.8	17.7	31
Melezitose	5.85	5.47	0.2	17.6	31
Inulin	3.26	3.21	0.1	12.8	31
Starch	4.82	3.01	0.1	12.4	31
Ethanol	7.83	4.08	0.7	17.3	30
Glycerol	8.43	3.62	0.9	14.8	31
Mannitol	7.86	3.71	2.4	15.6	31
Lactate	6.55	4.47	1.3	15.1	31
Succinate	6.40	3.36	2.1	18.1	31
Citraline	6.58	3.11	1.9	16.1	31
Control	32.70	8.16	15.4	48.5	31
Nitrate	7.48	5.51	0.1	16.8	31
Nitrite	4.99	2.37	1.0	10.7	31
Aspartic acid	6.81	6.37	0.1	25.0	31
ARSP	4.11	4.42	0.1	12.0	31
Glutamic acid	7.31	5.46	0.0	20.0	31
Ornit	23.70	8.95	0.7	40.5	31
Citrate	22.96	9.29	0.6	40.0	31
Org	27.43	7.39	14.7	39.5	31
W/O Vitamin	5.23	8.51	1.1	16.7	30
W/O Biotin	4.14	2.42	1.1	7.8	30
30°C	24.97	10.16	10.1	41.9	30
37°C	6.66	4.22	0.3	16.0	30
42°C	0.07	0.14	0.0	0.5	30

Table XXIII Hierarchical Cluster Analysis : Stage III

From Variable	To Z-score	Weighted Valid N
D-Glucose	ZD-Glucose	31
D-galactose	ZD-Galactose	31
L-Sorbose	ZL-Sorbose	31
Salicin	ZSalicin	31
Sucrose	ZSucrose	31
Maltose	ZMaltose	31
Melibiose	ZMelibiose	31
Cellebiose	ZCellebiose	31
Trehalose	ZTrehalose	31
Lactose	ZLactose	31
Raffinose	ZRaffinose	31
Melezitose	ZMelezitose	31
Inulin	ZInulin	31
Starch	ZStarch	31
Ethanol	ZEthanol	30
Glycerol	ZGlycerol	31
Mannitole	ZMannitole	31
Lactate	ZLactate	31
Succinate	ZSuccinate	31
Citraline	ZCitraline	31
Control	ZControl	31
Nitrate	ZNitrate	31
Nitrite	ZNitrite	31
Aspartic	ZAspartic	31
ARSP	ZARSP	31
Glutamic	ZGlutamic	31
Ornit	ZOrnit	31
Citrate	ZCitrate	31
Org	ZOrg	31
VFWO	ZVFWO	30
Glucose	ZGlucose	30
30°C	Z30°C	30
37°C	Z37°C	30
42°C	Z42°C	30

Fig. 8 Horizontal icecle plot using complete linkage (across number of clusters down case label and number)

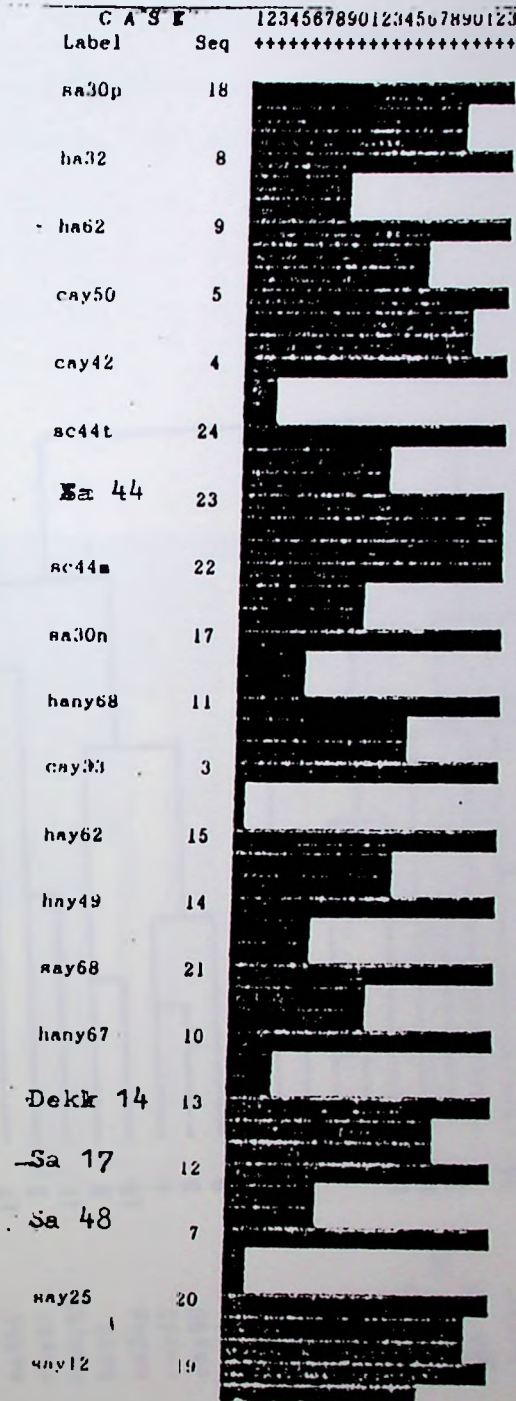


Fig. 10

Dendrogram using complete linkage

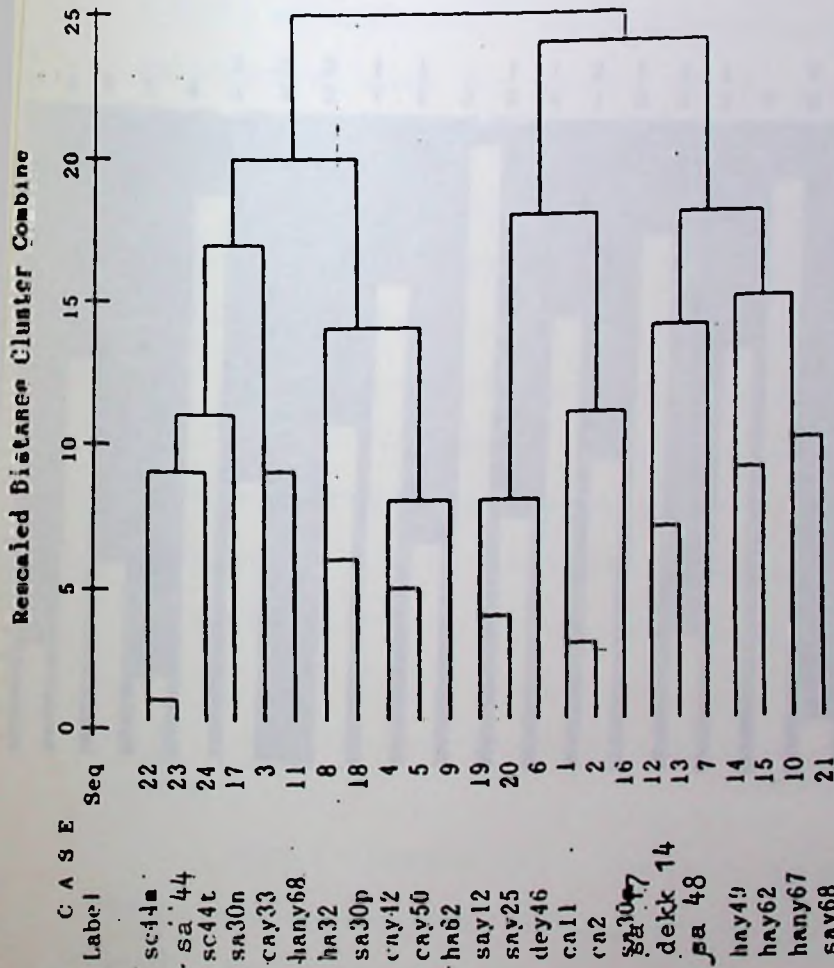


Fig. 9 Vertical icecle plot using complete linkage

SPSS/PC+

Vertical Icecle Plot using Complete Linkage

(Down) Number of Clusters (Across) Case Label and number

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	12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Results

Twenty three strains only were analyzed eight having been rejected. The complete linkage dendrogram (Fig. 10) shows that the 23 yeast strains analyzed grouped into 6 clusters at a similarity distance of $D \leq 5$ and 4 clusters at a distance of 4 or less. Five of the clusters formed at a distance of 10. These were the early fermenters Sc44T, Sa30M, Sa48, HanY67 and SaY68, as expected. Rapid growers CaY42, CaY50, Ha62 associated below a distance of 10, separate from the fermenters.

The association of Ha30P, CaY42 and Sa30P is rather curious, since Sa30P and Ha30P are generally good fermenters.

A similar situation is observed with Ca11, Ca2 and Sa30M. In general the numerical analysis has differentiated the fermenters from the biomass producers. In another experiment these two characteristics will be investigated further.

4.2 Approximate chemical analysis of Tonto

Introduction

Under traditional brewing techniques tonto deteriorates rapidly. Only after 48 hours the brew is sour. The brew is unpalatable due to rapid exhaustion of sugars and accumulation of acids and phenolic compounds.

RESULT: The study sought to answer questions on the feasibility of producing tonto as a wine, commercially sustainable in terms of quality.

METHODOLOGY

The tonto was prepared as usual (method described earlier), except that pure strains of yeasts were used and strict asepsis was observed. The strains were the 12 used before in the previous experiments.

Analysis

1. Sugar: Reading Brix by using the refractometer and then converting to percentage sugar using Tables (A,O,A,C).
2. Alcohol: Taking the specific gravity using the specific gravity bottles and converting to percentage ethanol from Table (A,O,A,C).
3. Calcium: Perchloric acid determined titrametrically using potassium permanganate digestion.
4. Missing: phenolic compounds.
5. Iron: Red off from the Atomic Absorptiometer, recorded in at X.

RESULTS

The results were recorded in Table XXIV.

There are no other techniques required to improve on the quality of tonto. The results of this investigation show that what is required is an initial high sugar content from bananas, that is no water should be added to the juice extract from properly ripened fruits, especially Kisubi, Kayinja, bogoya and sukari, used in combination. These observations follow from Pasteur Effect. In these circumstances, growth is virtually anaerobic and the sugars assimilated are metabolised to CO_2 and ethanol by the process of fermentation, until the sugar concentration has been reduced to a very low level of about 0.1%. Now under traditional brewing conditions, when the sugar concentration has been reduced to very low levels, the ethanol is re-utilised and is oxidised to CO_2 and water. The wine then tastes "bad". Presence of *Lactobacter* and *Acetobacter* further leads to wine (tonto) spoilage by converting the ethanol to lactic and acetic acids. The tonto becomes sour.

It is, therefore, imperative that the containers are kept as clean as possible to avoid heavy inocula of the bacteria.

If possible, selected yeast strains should be used to maintain good standards of banana wine quality.

Table XXIV Approximate chemical analysis of Tonto

Analysis	Dates of Sampling					
Sample	0	1	2	3	4	5
	23/8	24/8	25/8	26/8	2 weeks	5 weeks
Sugar %	20	19.75	19.5	9.5	9.5	8.0
Alcohol %	0	0	6.44	9.3	12.9	20.99
pH	4.8	5.02	-	5.03	4.7	3.75
<u>Acidity</u>						
Acetic acid % (W/V)	-	0.29	-	0.33	0.36	1.26
Calcium mg/ 100	3	9	1.8	-	2.8	0.026
Phosphorus ppm	-	0.03	0.019	-	0.025	0.026
Iron ppm	0.5	0.5	0.1	0.1	-	35
<u>Protein</u>	0.1	0.13	0.17	-	-	-

4.3 Influence of Tonto on yeast growth

Introduction

Tonto is a sweetish, semi-fermented banana juice mixed with roasted and ground sorghum (*Sorghum bicolor*) to give colour and flavour to the beverage. The bananas are a variety that is bitter when steamed and, therefore, not good for the traditional Kiganda dish called matooke. The beer bananas are of the *Musa sapiens* varieties called mbidde (Ganda) with genomes AAA, AAB and ABB triploids.

Researchers on physiology of micro-organisms have come up with several meanings for the word growth. Growth, as a metabolic process, may be considered as the irreversible increase in living substance in living organisms, by the synthesis of macromolecules. Living organisms can grow by increase in mass sometimes by cell division and an increase in cell numbers.

In fission or budding yeasts, increase in number is by growing until almost double their original size and then dividing into two. If environmental factors (temperature, nutrients, pH, etc.) are suitable the process is repeated so that all the yeast cells continue to grow and divide. Yeasts reproduce either by fission or budding. Their multiplication, therefore, corresponds to a geometric progression.

$$2^0 \text{ -----} > 2^1 \text{ -----} > 2^2 \text{ -----} > 2^n.$$

This process enables one cell to become successively, 1,2,4 etc, With no mathematical limit. Using simplifying assumptions that if cell divisions occur at equal time (t) intervals and all cell divide, with the same rates, and that none are dying, then the logarithm of cell number plotted against time will yield a straight line, giving the expression logarithmic growth.

$$\log N = \log N_0 + n \log 2 \quad (\text{a straight line}).$$

However, such situation as described above rarely exists in growth. If the total mass measurements are made on a culture of yeast cells which are growing, the base for logarithms is (e), and the cells are found to be growing exponentially. The values for the exponential function described a characteristic curve given by

$$y = ba^t.$$

There are two types. The growing exponential function when $a > 1$; and when $a < 1$ it is said to be a death exponential function.

After a given time of growth, growth begins to decrease. If t is the time in hours, after maximum growth is reached it appears that an exponential death of the type

$$y = ba^t$$

describes the situation, where a and b are constants to be determined from experimental data.

Cell death in yeasts may be defined as the irreversible loss of the ability of the cells to form colonies on a suitable medium. In many cases the rate of loss of viable cells at any one time has been found to be proportional to the number of viable cells. It therefore, seems reasonable to assume that death of cells follows first order reaction kinetics,

$$N = N_0 e^{-kt}.$$

The killing and death of yeasts are fundamental in brewing and baking and production of biomass and pharmaceutical. The process is also important in food preservation. Growth and death of yeasts will determine the amount of ethanol and biomass the cells will produce and can withstand or tolerate the environment during the process of fermentation or metabolism.

Initially tonto provides an ideal medium for yeast growth. Tonto is a sweetish medium, containing hexoses about 200 g/l. It contains amino acids and proteins, including a variety of mineral salts, such as calcium, phosphorus and a variety of vitamins (Table XXIV).

MATERIALS AND METHODS

Medium

Banana juice well mixed with roasted sorghum (*S. bicolor*).

Materials : six ripe clusters of each of Kisubi, Sukari, Kayinja (all of *Musa* varieties genome ABB); 20

kg of ripe fingers of each variety, were used for juice extraction; grass (*Imperata spp*) was used during squeezing or extraction of the juice, 5 kg of roasted and ground sorghum were used.

Equipment: Two large, clean sauce pans, 20 litre jerry cans, 500 ml mugs.

Labour: Two ladies were employed to squeeze juice from the ripe bananas; to roast and grind the sorghum.

Method: The ripe bananas with skins on were mixed with the grass in a large (20 litre sauce pan); juice was manually extracted out of the ripe bananas using grass to provide fibre; roasted and ground sorghum was added and the total extract of 40 undiluted fresh tonto was put into 2 jerrycans and the tonto allowed to ferment. The yeast flora naturally occurs on the banana fruits, as wild yeasts. The juice was allowed to ferment for 5 weeks at 23°C.

The aim of the above procedure was to simulate the traditional way of brewing tonto.

Yeasts were isolated, yeast number and biomass were recorded, approximate chemical analysis was done and results recorded. The parameters of the analysis included crude hexoses, proteins, amino acids, calcium, phosphate iron, ethanol, acetic acid and lactic acid and phenolic compounds. All the recordings were done at 0.24, 48, 72, 96, 120, 144, 168, 2 weeks, 5 weeks, on samples taken at these time intervals.

Total cell count was determined by enumeration using a haemocytometer. Viable counts (colony forming units, c.f.u.) involved counting the colonies produced by the viable cells on nutrient agar. Biomass was determined after centrifuging and washing with distilled water and drying at 100°C to constant weight in an oven, and the dry weight determined using an electronic balance. Dry weight was taken after 1,2,3,4 and 5 weeks. It was appreciated that this procedure was subject to systematic errors. However, these were the best means at hand. The errors originated from residual medium and drying. The errors were corrected using simplifying assumptions of an error not to exceed 2%. Hence, the error was given by $100 \times dx/x^2$ where x is the mass measured in mg. The method was essentially batch culture.

Method for yeast cell count using Neubauer haemocytometer

The haemocytometer consists of a special slide with a ruled area in the centre, a coverslip and a graduated pipette with a rubber tubing. Under low power, X 100 magnification, the central part is ruled. The middle of the ruled area consists of a grid 1 mm^2 in area. This is usually called TYPE-A SQUARE (See Figure 11 A-C).

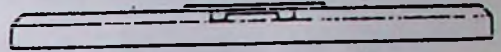
Type-A square is subdivided by lines into 25 TYPE-B Squares, each of which has an area of $1/25 \text{ mm}^2$.

Fig. 11 Neubauer haemocytometer slide

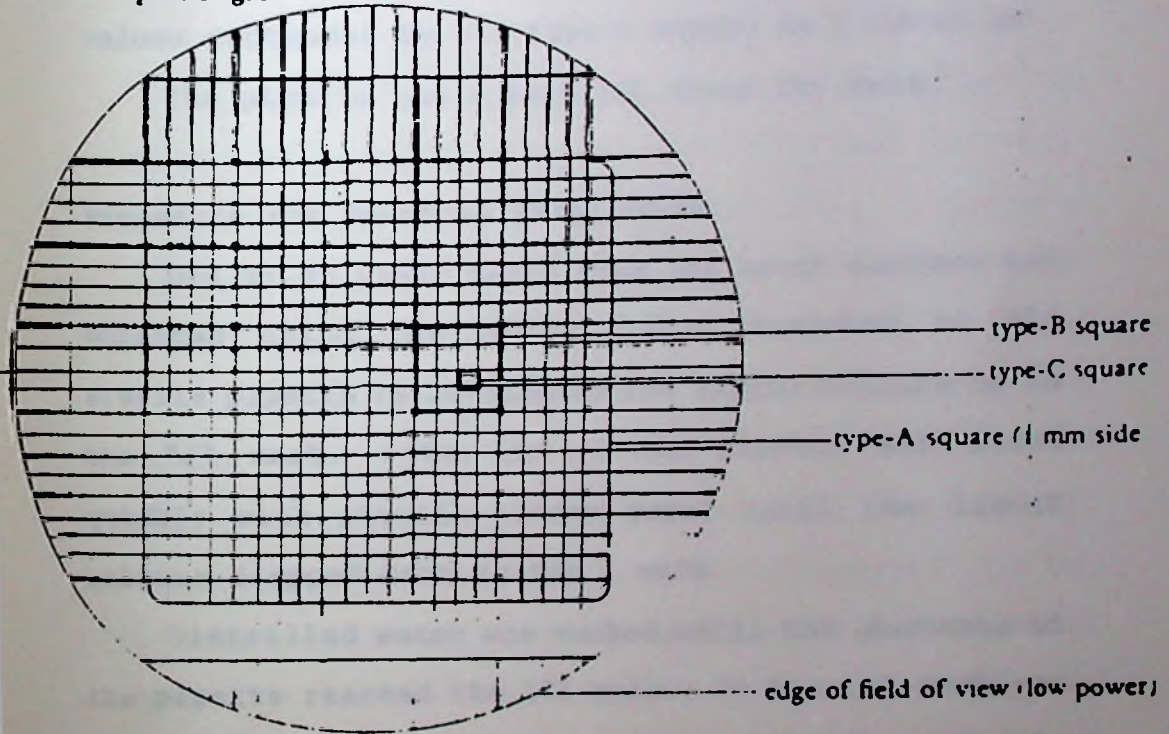
A Surface of slide



B Section of slide showing coverslip in position



C Central part of grid



Each type-B square is further subdivided by single lines into 16 TYPE-C squares which have an area of $1/400 \text{ mm}^2$.

The surface of the slide between the two deep grooves is 0.1 mm lower than the rest of the slide on either side of the grooves. So when the coverslip is put on, its lower surface clears the ruled surface of the slide by 0.1 mm. The volume subtended by the type-A square is therefore 0.1 mm^3 . The volume subtended by a type-B square is 0.004 mm^3 , and the volume subtended by the type-C square is 0.00025 mm^3 .

The pipette has a mark 101 above the bulb.

Procedure for counting yeast cells

One ml of yeast cells from the batch culture was obtained using the rubber tubing attached to the sterile pipette by sucking up the liquid culture up to the "1" mark. The tip of the pipette was dried quickly with sterile filter paper until the liquid culture dropped back to the 1 mark.

Distrilled water was sucked until the contents of the pipette reached the 101 mark. If the 101 mark was passed, the pipette had to be emptied and the procedure started again. Care was taken not to contaminate the culture during the process.

The rubber tubing was then removed from the pipette and the two ends of the pipette closed for at least a minute. With the culture thoroughly mixed,

six drops were blown out so as to expel excess water. The yeast culture was then diluted to 1 in 100.

The coverslip was placed in the centre of the slide. One drop of diluted yeast culture from the pipette was put onto the slide alongside the coverslip in the area between the two deep grooves. The yeast culture was drawn under the coverslip by capillary action. Five minutes were allowed for the yeast cells to settle. If the yeast culture flowed into the grooves, the slide was cleaned and another drop put on.

The slide was placed under the microscope the illumination adjusted so that the grid and the yeast cells could be clearly seen. If the yeast cells were unevenly distributed the slide was cleaned and the process repeated.

The yeast cells in 100 type-C squares were counted and recorded. It was noted that in each square all the cells which lay entirely within it, plus those which were touching or overlapping the top and left-hand sides were the only ones that were counted. Those touching or overlapping the bottom and right-hand sides even if they were within the square were not included.

The mean number of yeast cells in type-B square was calculated. Bearing in mind the volume represented by type-C square, and the dilution factor,

the number of yeast cells per ml in the culture was then calculated and the results recorded.

RESULTS

The results were used in the Batch Culture yeast growth isolated from tonto (Figs. 12 and 13).

The results show an initial slow growth rate up to a peak after 40 hours. Thereafter the count declined to below 8 after 480 hours.

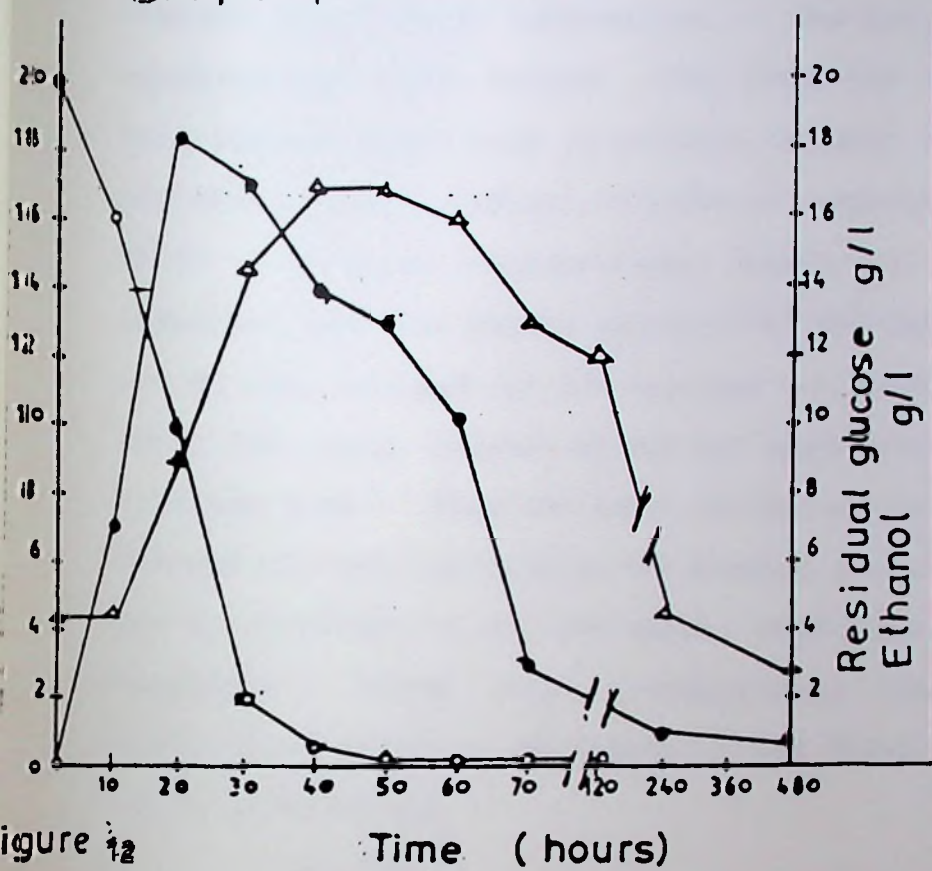
After 5 weeks the tonto contained less than 0, 1% crude hexoses .. protein 0 amino acids 0 calcium 5 mg/100 phosphate 0.03 ppm iron 35 ppm ethanol, total acid 0.8% and pH 5.3. The methods used for these analyses are those described under approximate chemical analysis of tonto (Table XXIV).

The results in Figure 11 give the situation after 20 days of growth and fermentation.

The growth patterns of yeasts, c.f.u. are plotted in Fig. 12. Colony forming units rise from about 16 to more than 60 after 40 hours. Thereafter the c.f.u. decline to about 8 after 480 hours. Meantime, ethanol increases sharply from 0 to over 18 g/l within 24 hours of fermentation. Thereafter ethanol decreased to about 1.2 g/l after 480. The rapid rise in ethanol is paralleled with a sharp fall in sugar concentration, so that after only 40 hours the sugar level is less than 0.1 g/l. Fig. 13 shows percentage survivors of yeasts. There is a rapid, exponential, death of

Fig. 12 Yeast growth in Batch Culture

Fig. 13 Percentage of survivors of yeasts

YEAST GROWTH IN
BATCH CULTURE

▲ c.f.u
 ○ residual glucose
 ■ ethanol

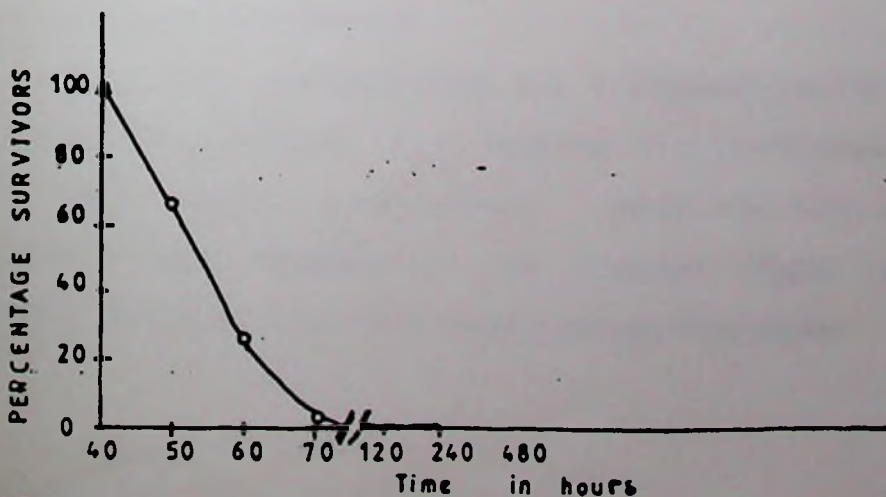


Fig 13

yeast cells after 40 hours of fermentation. The cells are nearly all killed by 120 hours of fermentation.

Batch culture methods rely on altering the overall nutritional composition of the medium or by changing the sugar source. The investigation starts with banana juice rich in hexoses, mineral salts and proteins, with no ethanol or phenolic compounds (Table XXIV). In these circumstances, growth is virtually anaerobic and the sugars assimilated are metabolised to CO_2 and ethanol by the process of fermentation, until the sugar concentration has been reduced to a very low level. When the sugar concentration has been reduced to very low levels, the ethanol is re-utilised and is oxidized to CO_2 and water, producing phenolic compounds. Under these circumstances yeast cells start dying rapidly, according to the first order of reaction kinetics,

$$N = N_0 e^{-kt}.$$

The killing rate was dependent on a number of factors in the fermenting banana juice. Some of the factors included depletion of nutrients and accumulation of toxic phenolic compounds.

Since the investigation was a simulation of the traditional method of tonto brewing, the technique did not take aseptic precautions. Much of the acid produced and degradation of ethanol might have involved lactic acid and acetic acids bacteria.

4.4 Ethanol tolerance in yeasts isolated from tonto, ethanol and biomass yield

Introduction

The feasibility of using selected pure strains of yeasts from tonto for high yield of ethanol and low yield of biomass under batch culture conditions was investigated.

The twelve selected yeast strains produce ethanol from the fermentation of glucose, a major component of banana juice, Purseglove (1991). Tonto brewers depend on a mixed wild population of yeasts for the fermentation.

This aspect of the biology of yeasts isolated from tonto has not been studied beyond the practical aspect of banana wine production by a few adventurous Ugandans. During study of yeasts growth in batch culture, it was noted that many yeast strains could not survive at 14% ethanol for long periods of storage. This is often the problem experienced by cottage tonto brewers in Uganda.

Thus at the outset of the study it was thought that high ethanol yields and tolerance by yeast cells might be useful characteristics to the prospective banana wine producer.

MATERIALS AND METHODS

Organisms (yeasts were strains isolated from tonto during previous exercise as described earlier).

The strains were SaY30, SaY21, SaY25, HanY68, Sc44, DeY46, DeY48, HaY17, ScY26, Ca2, Call and HanY62.

Media: Glucose peptone agar (GPA) contained 2% (w/v) Bacto Peptone and 2% (w/v) Bacto Agar. Glucose peptone broth (GPB) had the same constituents without agar. Yeast Nitrogen Base without amino acids (YNB) was prepared in accordance with the manufacturers' instructions.

Methods

Ethanol tolerance: Cells of yeast strains were grown in 50 ml YNB with 1% glucose contained in 300 ml nephelo flasks with side arms to control evaporation. Ethanol of at concentrations of 1,2,5,10,14% (v/v) was added to the flasks at the time of inoculation. Cells for study were grown on GPA at 23°C for 24 hours, harvested and washed twice in sterile water. Growth was initiated at a cell concentration of about 5×10^6 cells ml^{-1} (by serial dilution). The cultures were grown in three litre sterile containers at 23°C and growth was assessed at regular intervals as follows:

- (a) Growth was measured by direct method of dry weight. After drying to constant weight at 100°C in an oven, dry weight was determined by weighing on an electron balance.

- (b) Determination of ethanol yield by the selected yeast strains: Cells of the selected strains were grown in yeast extract peptone broth, for ethanol fermentation with 2% glucose at 23°C. The level of ethanol was determined using an alcoholometer over a period of 72 hours.

RESULTS

Table XXV Ethanol Tolerance (growth in mg after 72 hours)

Ethanol (%)	1%	2%	5%	10%	14%
Strain					
DeY46	14.6	14.2	10.4	8.3	5.1
DeY48	11.0	7.3	7.0	6.4	5.0
SaY21	10.8	10.0	10.0	8.7	8.0
SaY25	8.0	6.0	3.0	0	0
Sc44M	6.4	4.2	2.5	1.7	1.0
HanY68	3.0	1.0	0	0	0
HaY17	3.4	0.09	0	0	0
ScY26	0.7	0	0	0	0
SaY30	0.6	5.0	3.1	0	0
HaY62	12.0	10.1	10.0	0	8.2
Ca2	10.7	9.8	8.0	8.0	7.6
Call	13.5	12.3	12.0	12.0	11.1

Table XXVI Ethanol and biomass yield (after 72 hours)

Strain	Ethanol yield (%)	Biomass yield (mg)
DeY46	3.1	0.2
DeY48	2.2	0.4
SaY21	4.3	0
SaY25	4.1	1.2
Sc44M	3.4	1.7
HaY68	1.0	1.0
HaY17	1.0	0.4
ScY26	1.1	3.0
SaY30	4.1	1.0
HaY62	3.3	9.0
Ca2	1.2	12.8
Call	1.3	14.1

Twelve strains of yeasts were inoculated in triplicate into flasks of Yeast Nitrogen Base with 1,2,5,10 and 14% ethanol and the results recorded in Table XXV. Values obtained for ethanol tolerance revealed that only 6 of the 12 yeast types could survive at 14% ethanol concentration. These included DeY46, DeY48, SaY12, Ca2, Call and HaY62. The others, SaY25, Sc44M, HanY68, HaY17, ScY26 and SaY30 hardly survived 5% ethanol concentration.

Production of ethanol and biomass on glucose 20 g/l cultured for 72 hours: Values obtained for ethanol yield and biomass yield are recorded in Table XXVI. The results demonstrate that most of the yeasts fermented glucose. The mean ethanol yield was 2.5

with standard deviation of 1.34. Biomass yield had a mean weight of 3.7 mg and standard deviation of 5.2 mg. There was relatively more variability in biomass yield among the 12 selected yeast strains than in ethanol yield.

Storage of fermented tonto is a problem to the cottage tonto brewer in Uganda. Tolerance of ethanol among tonto yeasts is, therefore, an important characteristic in brewing industry. Stimulation or inhibition of growth or killing or death of yeast cells in tonto were investigated. Killing and death in yeasts were defined as the irreversible loss of ability to grow and reproduce as it related to experimental conditions outlined in the methodology. The term killing or death related to the loss of colony forming ability. Quantitative estimation of growth, killing and death in yeasts isolated from tonto were measured. The killing rate was dependent on the ethanol concentration in the fermenting medium. The results show that strains DeY46, DeY48, SaY21, Ca2 Call and HaY62 were highly tolerant of ethanol in comparison to strains SaY25, Sc44M, HanY68, HaY16, ScY26, and SaY30. These could be said to be poorly tolerant by the standards of Casey & Engledw (1986).

It was observed that 5% ethanol wiped out HaY68, HaY17 and ScY26. Growth of Sc44M was reduced by more than 50% at 5% ethanol concentration. Ethanol

tolerance might be genetically controlled. This needs to be investigated by controlled crosses and selection.

The production of ethanol and biomass by 12 selected yeasts was investigated and the results recorded in Table XXVI. The mean ethanol yield was 2.5 and standard deviation 1.34 in 72 hours. The values for biomass were a mean yield of 3.7 mg with standard deviation of 5.2 mg. This shows that yeasts isolated from tonto are largely fermentative and should be exploited more for ethanol yield with the significant exception of Ca2 and Call, with respective yields of 12.8 and 14.1 mg.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Some traditional alcoholic beverages and their importance in Ugandan communities

Many indigenous fermentation products, either in form of beer (tonto, kwete, Muramba or Amarwa) or in form of bread or pastes, or porridge, provide essential food values to the consumers in form of proteins, amino acids, vitamins and calories.

However, Hasseltine (1965) has observed that preparation of foods by fermentation with yeasts has certain disadvantages. Any additional processing results in additional time, equipment and labour costs. There is loss of nutrients during washing in preparation for fermentation. The yeasts used in fermenting the substrate will require energy for growth. About 5% of the substrate will be metabolised. Under no circumstances is there any increase in substrate apart from biomass yield of yeast cells. The question then arises as to why the fermentation of foods is used on such wide scale in Uganda and other parts of the world where food is in short supply? It should be appreciated here that millet and sorghum together with cassava and beer bananas, because of drought resistance are crops *per excellence* for dry regions and areas with unreliable rainfall. Without these crops many parts of Uganda would be waste land. These crops in their natural

form, however, are not very palatable. During the process of steeping detoxication of bitter cassava takes place. After drying and milling followed by fermentation, mixed with sorghum or millet during the preparation of kwete, muramba or amarwa/ajon, the bitter chemicals in the cassava have completely disappeared. During malting or germination, drying and milling of sorghum or millet, followed by fermentation these grains gain excellent taste and large quantities of the grain beverages are consumed in Uganda. The bananas used in tonto preparation are always the bitter varieties. These bananas are not used for preparing the traditional kiganda dish called *matooke* because of their bitterness. These bitter bananas are now becoming ubiquitous in Buganda and many other parts of Uganda where, owing to poor soil management, the *matooke* cannot grow. The ripe beer bananas are very sweet, with a lot of sugar, about 200 g litre of juice (Perceglove, 1991). During preparation of these fermentation products certain undesirable odours and bad flavours are either destroyed or masked by yeast metabolites such as ethanol or other aromatic compounds.

A factor which cannot be underestimated is the monotonous diet of much of Ugandan communities where protein in the form of meat and dairy products is not available. The people's foods lack flavour. Usually materials of plant, animal, mineral or synthetic

origin are added to foods to make them more attractive or more palatable and appealing to the taste. People in Uganda, because of their poor economics, cannot afford appetizers. Fermentation adds to the flavour of the food through production of ethanol, aromatic substances and detoxication or destruction of bitter and musky materials in the traditional food materials such as sorghum, millet or cassava or bananas.

5.2 Ecology of yeasts isolated from fruits, grains and beverages

This study has not been merely a listing of yeast strains, present on fruits, grains and in beverages. In other words, the list is not an end in itself. It was of interest to investigate the active life of yeast strains in their environment and perhaps suggest practical application of the characteristics shown by the yeast strains.

From Tables II to IV the following picture emerges: In Table II at least 66% of the fermentative strains were isolated from ripe bananas. The next 'habitat' was kwete, with 21%. The rest of the strains were distributed more or less equally among other fruits, except for mangoes where a remarkable 15% of the isolates came from the surfaces of mangoes. Interestingly, the relative number of isolates from

tonto were low, this is in terms of biodiversity. Temperatures in tonto quite often were recorded above 42°C.

Perhaps the temperature factor tended to eliminate mesophiles whose optimum temperatures is 28 to 37°C. Ripe bananas, on the other hand, emit aromatic odours and exude sweet juices through their surfaces. These juices and aromatic odours attract swarms of flies (*Drosophila*, *Musca*), butterflies and bees. These insects feed on the exudates from the banana surfaces. These animals must be responsible for the inoculation of banana fruits with yeasts. On plating, the yeast colonies appeared within 18 hours, similar to growth rate of colonies developing from active cultures. This implies that the yeast cells must have been active on ripe banana surfaces. The ripe mango fruit is also known to exude sweetish juices and to emit aromatic odours. The relatively high number of isolates from ripe mango surfaces can also be accounted for in a similar manner. On the other hand, passion fruits, pawpaws and pineapples are not known to exude any sweet or nutritive juices, although they emit aromatic odours. The lack of nutrients on the surfaces of these fruits might account for the relatively low count of yeast isolates.

Another interesting case is that of kwete. Kwete is very rich in nutrients, from malted millet, sorghum

and cassava flour. The brew is quite sweet and attracts swarms of flies and bees during brewing processes, which may be responsible for the relatively high population density and biodiversity of yeasts. A rather similar picture emerges in yeasts isolated from Mbarara/Rukungiri.

In Mbarara/Rukungiri yeasts isolated from bananas accounted for 55.8%, mangoes for 22% and very low counts from tonto. Interestingly, the count was quite high from Muramba (17.9%). Muramba is similar to kwete in composition, except that more sorghum is used in the preparation of the brew.

In the Jinja/Tororo/Mbale region the picture was different. Most of the yeast isolated came from amarwa and grains. There were virtually no yeasts isolated from fruits. Amarwa showed a very high proportion of isolates, averaging 63.8% of the total number of isolates. Amarwa, muramba and kwete are generally rich in nutrients and the process for their preparation is always associated with flies and inocula from previous brews.

Fermentative yeasts contributed at least 60% of the yeast flora isolated from the ripe banana fruits. This may be explained by the fact that fermentative yeasts are quick growers and tend to be isolated early. Warcup (1960) points out that dilute plates always record only a part of the species present on surfaces, or from other extracts of fluids.

It can be asserted that the fresh beverages which contain rich sources of nutrients, such as sugars, proteins, vitamins and mineral salts gave the highest frequencies of cell forming units. However, as far as biodiversity was concerned, banana fruits gave the highest count of yeast strains. The following gives a picture on the biodiversity.

Bananas (ripe): *Candida* Y50, *Candida* Y60, *Candida pulcherrima*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Hanseniaspora* Y68, *Hanseniaspora* Y20, *Saccharomyces* Y12, *Saccharomyces* Y21, *Schizosaccharomyces* Y26, *Cryptococcus* Y37, *Debaryomyces* Y13, *Schizosaccharomyces pombe*, *Saccharomyces pastorianus*, *Torulopsis* Y29, *Hansenula* Y49, *Hansenula* Y62, *Lodderomyces elongisporus*, *Saccharomyces chevalieri*, *Dekkera bruxellensis*, *Saccharomyces* Y56, *Debarymces* 10. The mean count of colony forming units for fruits in general was 74.4 cm^{-2} .

Tonto : The following were isolated from tonto. *Candida* Y6, *Dekkera bruxellencis*, *Hansenula* Y17, *Saccharomyces pastorianus*, *Saccharomyces ellipsoides*, *Hanseniaspora* Y68, *Saccharomyces* Y21, *Saccharomyces* Y12, *Sachizosaccharomyces* Y26, *Cryptococcus* Y50, *Saccharomyces* Y25.

Muramba : *Candida* Y42, *Hansenula* Y49, *Hansenula* Y62, *Hansenula* Y67, *Saccharomyces* Y30, *Saccharomyces* Y68, *Sachizosaccharomyces* Y56, *Debaryomyces* Y46, *Debaryomyces* Y48, *Saccharomyces pastorianus*.

Amarwa : *Candida* 2, *Candida* 11, *Hansenula* 62, *Hansenula* 32, *Schizosaccharomyces* 30M, *Saccharomyces* 30N, *Saccharomyces* 30P, *Schizosaccharomyces* 44M, *Schizosaccharomyces* 44N, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Schizosaccharomyces* 44T. The colony forming units counted for beverages in general was $5 \times 10^7 \text{ ml}^{-1}$.

5.3 Physiology of yeast strains isolated from fruits, grains and beverages

Approximate chemical analysis during 'maturation' of tonto

Under traditional brewing techniques tonto deteriorates rapidly. Only after 48 hours, the brew is sour. The brew is unpalatable due to rapid exhaustion of sugars and accumulation of acids and phenolic compounds. However, there are techniques that have been shown to be essential in the production of 'good' tonto. The results from this investigation show that what is required is an initial high sugar content from ripe bananas. That is, no water should be added to the juice extracted from properly ripened fruits, especially the varieties *Kisubi*, *Kayinja*, *Bogoya* and *Sukali*, used in combination. These observations follow from Pasteur Effect. In these circumstances metabolism is virtually anaerobic and sugars assimilated are metabolised to carbon dioxide and ethanol by the process of fermentation until the

sugar concentration has been reduced to a very low level of about 0.1%. When the sugar concentration has been reduced to very low level the ethanol is re-utilised and is oxidised to carbon dioxide and water, also producing phenolic compounds. The wine then tastes "bad". Presence of *Lactobacter* and *Acetobacter* further leads to the wine (tonto) spoilage by converting the ethanol to lactic acid and acetic acid. The tonto becomes sour. It is, therefore, imperative that the containers are kept as clean as possible to avoid heavy inocula of the spoilage bacteria. If possible, selected yeast strains be used to maintain good standards of banana wine quality.

5.4 Influence of tonto on yeast growth

During the study a total of 12 yeast strains were selected. These included *Saccharomyces cerevisiae*, *Dekkera bruxellensis*, *Debaryomyces* Y46, *Saccharomyces* Y21, *Saccharomyces* Y25, *Schizosaccharomyces* 44M, *Hansenula* Y68, *Schizosaccharomyces* Y26, *Saccharomyces* Y30, *Hansenula* Y62, *Candida* 2 and *Candida* 11.

With an initial 20% sugar, 4.3% alcohol, 3 mg/100 calcium, 0.0 ppm phosphorus 0.5 ppm iron, 0.0% phenolic compounds, 0.1% protein, 0.0% (w/v) acid, pH 4.8, the composition of the batch culture after 5 weeks had altered as follows: sugar 8.0%, alcohol 20.99%, pH 3.75, acid 1.26% (w/v), calcium 5%, phosphorus 0.026 ppm, iron 35 ppm, protein 0.0%,

phenolic compounds 10%. Colony forming units rose from about 16 to more than 60 mean count after 40 hours of fermentation. Thereafter the colony forming units decline to about 8 after 480 hours. There was a corresponding, almost exponential death rate of yeast cells after 40 hours of fermentation. There was virtually no living yeast cell after 5 weeks. Although the sugar content was high, initially, there was no control of contaminants such as bacteria. The idea of the batch culture was to simulate conditions that prevail during fermentation procedures and processes in the traditional brewing industry.

Batch culture methods rely on altering the overall nutritional composition of the medium or by changing the sugar source. The investigation starts with banana juice rich in hexose sugars, mineral salts and protein with no ethanol, no phenolic compounds. In these circumstances metabolism is virtually anaerobic and the sugars are assimilated and metabolised to carbon dioxide and ethanol by the process of fermentation, until the sugar concentration has been reduced to a very low level. When the sugar concentration has been reduced to very low levels the ethanol is re-utilised and is oxidized to carbon dioxide, water, producing phenolic compounds in the process. Under these circumstances yeast cells start dying rapidly, according to the first order of reaction kinetics

$$N = N_0 e^{-kt}$$

where N = the final cell number (or final population)
of yeasts

N_0 = the initial number of cells or initial
population

e = the exponential function base

k = $\ln N_0$, the exponent to e

t = time

the negative sign on the exponent k indicates the
process of death of yeast cells.

The killing rate was dependent on a number of
factors in the fermenting banana juice. Some of the
factors included depletion of nutrients and
accumulation of toxic phenolic compounds.

Since the investigation was a simulation of the
traditional method of tonto brewing, the technique did
not take aseptic precautions. Much of the acid
produced and the degradation of the ethanol might have
involved lactic acid and acetic acid bacteria.

5.5 Ethanol tolerance in yeasts isolated from tonto, ethanol and biomass yield

The feasibility of using selected pure strains of
yeasts from tonto for high yield of ethanol and low
yield of biomass under batch culture conditions was
investigated, again using the 12 yeast strains
discussed earlier.

Storage of fermented tonto is a problem to the cottage brewer in Uganda. Tolerance of ethanol among tonto yeasts is, therefore, an important characteristic in brewing industry. Stimulation or inhibition of growth or killing or death in yeasts is defined as the irreversible loss of ability to grow and reproduce as it relates to experimental conditions outlined in the experimental methodology. The term killing or death related to the loss of colony forming ability. The killing rate was dependent on the ethanol concentration in the medium. The results show that strains of *Debaryomyces* Y46, *Dekkera bruxellensis*, *Saccharomyces* Y21, *Candida* 2, *Candida* 11 and *Hansenula* Y62 were highly tolerant of ethanol in comparison to strains *Saccharomyces* Y25, *Schizosaccharomyces* 44N, *Hansenula* Y84, *Hansenula* Y16, *Schizosaccharomyces* Y26, *Saccharomyces* Y30. These could be said to be poorly tolerant by the standards of Casey and Englew (1986). It was observed that 5% ethanol wiped out *Hansenula* Y68, *Saccharomyces pastorianus*, *Schizosaccharomyces* Y26. Ethanol tolerance might be genetically controlled. This needs to be investigated by controlled crosses and selection for the benefit of the brewing industry in Uganda.

The results also show that yeast isolated from tonto are largely fermentative and should be explored

more for ethanol yield with the significant exception of Ca₂, and Ca₁₁, which were exclusively oxidative for biomass yield.

Finally, it was pointed out earlier that this study has not been merely a listing of yeast strains present on surfaces of fruits, grains and beverages. It is of interest to investigate the active life of yeast strains in their natural environment. A few ideas have evolved from this study. These have been put across under the ecology of yeasts discussed before.

However, yeasts grown axenically (that is yeast cultures of a single species of yeast) on a single sterilised medium are unlikely to behave in the same way as they would in a complex environment such as those prevailing on surfaces of fruits, grains or in beverages such as tonto, muramba or amarwa, where other organisms, such as bacteria and moulds are present which compete for available nutrients and liberate compounds such as toxins and antibiotics and where resources are continually being reduced to zero, within a closed system.

Sterilisation of artificial media may further complicate interpretation of the results, in ecological terms, by chemically altering the medium. It would be difficult to envisage the 'natural' behaviour of yeasts from results obtained through plating on artificial media. In addition to these

problems of environment and nutrition on yeast behaviour, and hence on yeast characterisation, is a basic difficulty of measurement of growth upon which yeast taxonomy is based. Yeasts have extensive adaptability determined by their small size. A cell has space for only 100,000 molecules of protein (Hans, 1986). Enzymes that are not currently being used cannot be held in reserve and therefore, certain catabolic enzymes are produced only if the appropriate substrate is present. Such inducible enzymes can constitute up to 10% of cellular protein under some conditions (Hans, 1986).

5.6 Conclusions

As a solution to the almost flux state of yeasts it was generally considered best to compare yeast strains over a rigidly standardised set of tests. It was thus appreciated that certain of these conditions would introduce bias when measuring the degree of similarity between pairs of yeasts during characterisation. To counteract these problems the following strategies were adopted:

1. There was regular monitoring of morphological characteristics of yeasts growing on fruit grains and in beverages and those characteristics on artificial media. These characteristics were always constant for both media or environments.

2. For physiological characterisation, a large number of criteria were used. Over 70 criteria were used for yeast characterisation. Each character was given an equal weight during the analysis. These characteristics effectively narrowed down the possible classification of an unknown yeast strain. A solution to the almost flux state of yeasts it was considered best to compare yeasts strains over a rigidly standardised set of tests. According to Hughes (1978); if a wide range of characteristics are observed any bias should be "diluted" out. In this study at least 70 characters (cultural, morphological and physiological) were used.

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APPENDIX I

KEY TO CODES: A TAXONOMIC STUDY OF YEASTS

LOCALITY = V_{ij} (V_i = District
CODE

V_j = village)

SAMPLES = $S_y Y_i$ (Y_i = yeast strain)

Masaka/Rakai $V_{ij} = MR_{ij}/MR_{2j}$
 $MR_{ij}/MR_{2j} B_y Y_i$

MR_{11} = Kasasa MR_{21} = Kabonera
 MR_{12} = Masaka MR_{22} = Bukunda
etc.

Banana = B_y

Tonto = TO

^g
Sorghum = SO

Millet = MI

Maize = MAI

i.e., $S_y = B_y$
 y = variety

Kwete = KW

Pineapple:

$S_y = P$

Mango:

$S_y = M$

Orange:

$S_y = O$

etc.

- do -

Bushera = BU

- do -

Muramba = MU

- do -

Amarwa = MA

- do -

- do -

Ajon = MA
Kwete = KW

- do -

Mbarara/Rukungiri =
 $MRU_{ij}/MRU_{2j} BUY_i$
 $V_{ij} = MRU_{ij}/MRU_{2j}$

Jinja/Tororo =
 $V_{ij} = TJ_{1j}/TT_{2j}$

Mbale/Soroti =
 $V_{ij} = MS_{1j}/MS_{2j}$

APPENDIX II Table XXIX

Composition of chemically defined media¹ for taxonomic study of yeasts

Formula ingredients per litre	Bacto-yeast morphology agar	Bacto-yeast nitrogen base for assimilation tests	Bacto-yeast carbon base for nitrogen assimilation tests	Bacto-vitamin free yeast base for vitamins requirement test
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Nitrogen sources:

Ammonium sulphate	3.5 g	5 g	none	5 g
Bacto-asparagine	1.5 g	none	none	none

Carbon source:

Bacto-dextrose	10 g	none	10 g	10 g
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Amino acids:

L-Histidine monohydrochloride	10 mg	10 mg	1 mg	10 mg
DL-Methionine	20 mg	20 mg	2 mg	20 mg
DL-Tryptophane	20 mg	20 mg	2 mg	20 mg

Vitamins:

Biotin	20 µg	20 µg	20 µg	none
Calcium pantothenate	2000 µg	2000 µg	2000 µg	none
Folic acid	2 µg	2 µg	2 µg	none
Inositol	10000 µg	10000 µg	10000 µg	none
Niacin	400 µg	400 µg	400 µg	none
p-aminobenzoic acid	200 µg	200 µg	200 µg	none
Difco				

Table XXIX Cont..

Formula ingredients per litre	Bacto-yeast morphology agar	Bacto-yeast nitrogen base for assimilation tests	Bacto-yeast carbon base for nitrogen assimilation tests	Bacto-vitamin free yeast base for vitamins requirement test
Pyridoxine hydrochloride	400 µg	400 µg	400 µg	none
Riboflavin	200 µg	200 µg	200 µg	none
Thiamine hydrochloride	400 µg	400 µg	400 µg	none
Compounds supplying trace elements:				
Boric acid	500 µg	500 µg	500 µg	500 µg
Copper sulphate	40 µg	40 µg	40 µg	40 µg
Potassium iodine	100 µg	100 µg	100 µg	100 µg
Ferric chloride	200 µg	200 µg	200 µg	200 µg
Manganese sulphate	400 µg	400 µg	400 µg	400 µg
Sodium molybdate	200 µg	200 µg	200 µg	200 µg
Zinc sulphate	400 µg	400 µg	400 µg	400 µg
Salts:				
Potassium phosphate monobasic	0.85 g	0.85 g	0.85 g	0.85 g
Potassium phosphate dibasic	0.15 g	0.15 g	0.15 g	0.15 g
Magnesium sulphate	0.5 g	0.5 g	0.5 g	0.5 g
Sodium chloride	0.1 g	0.1 g	0.1 g	0.1 g
Calcium chloride	0.1 g	0.1 g	0.1 g	0.1 g

Formula ingredients per litre	Bacto-yeast morphology agar	Bacto-yeast nitrogen base for assimilation tests	Bacto-yeast carbon base for nitrogen assimilation tests	Bacto-vitamin free yeast base for vitamins requirement test
Bacto agar	18 g	none	none	none
Amount of final medium from 100 g dehydrated medium	2.81	14.91	8.51	5.91
Amount of dehydrated medium per 1 t. of finished	35.0 g	6.7 g	11.7 g	

1) These media are marketed by the Difco Laboratories of Detroit (Michigan) in dehydrated form. They are based on the formulae published by WICKERHAM (1951) but since then have been fortified with greater amounts of biotin, inositol and calcium pantothenate.

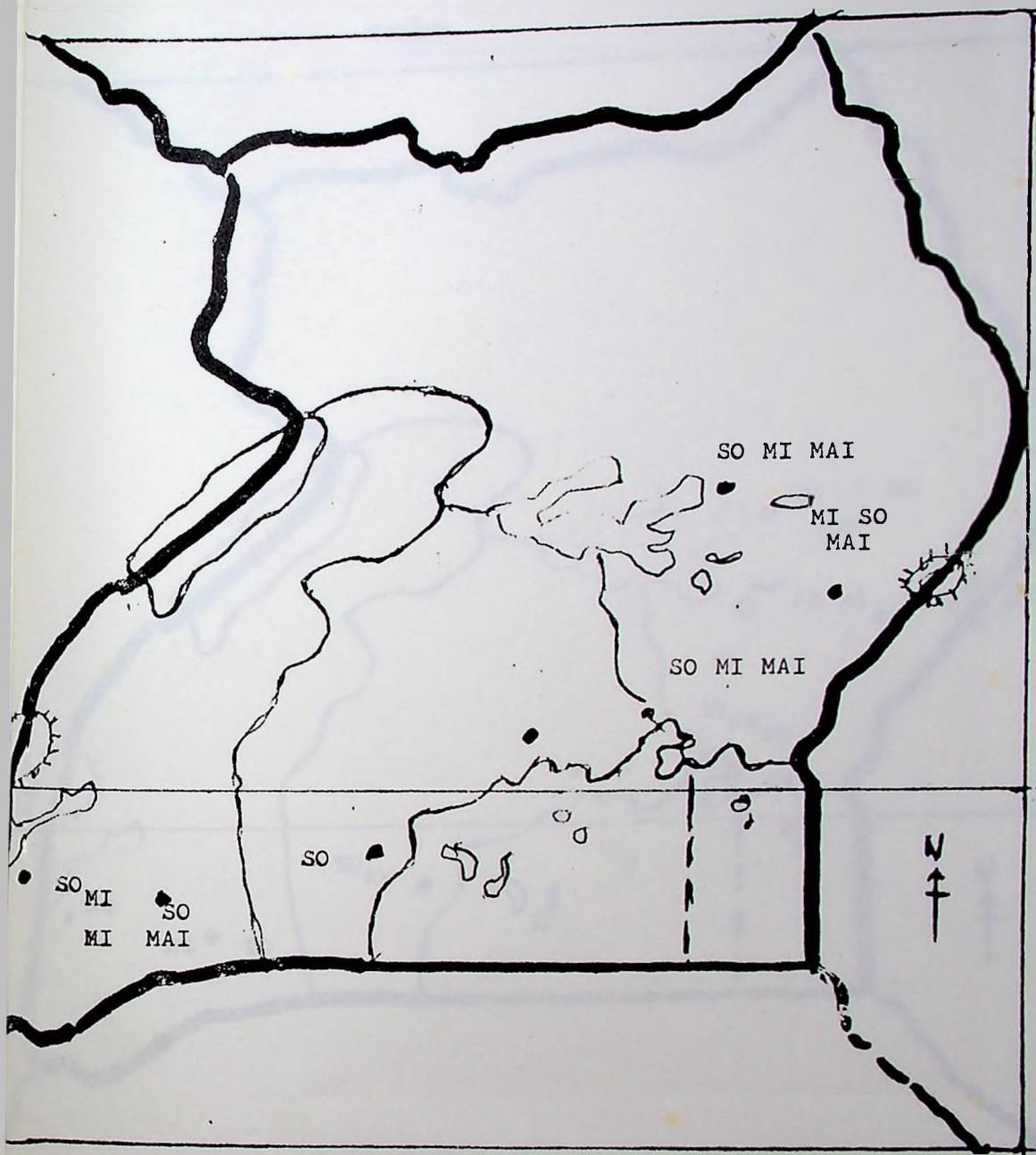
APPENDIX III Sampling Localities

Fig. 14 Distribution map for Fruits



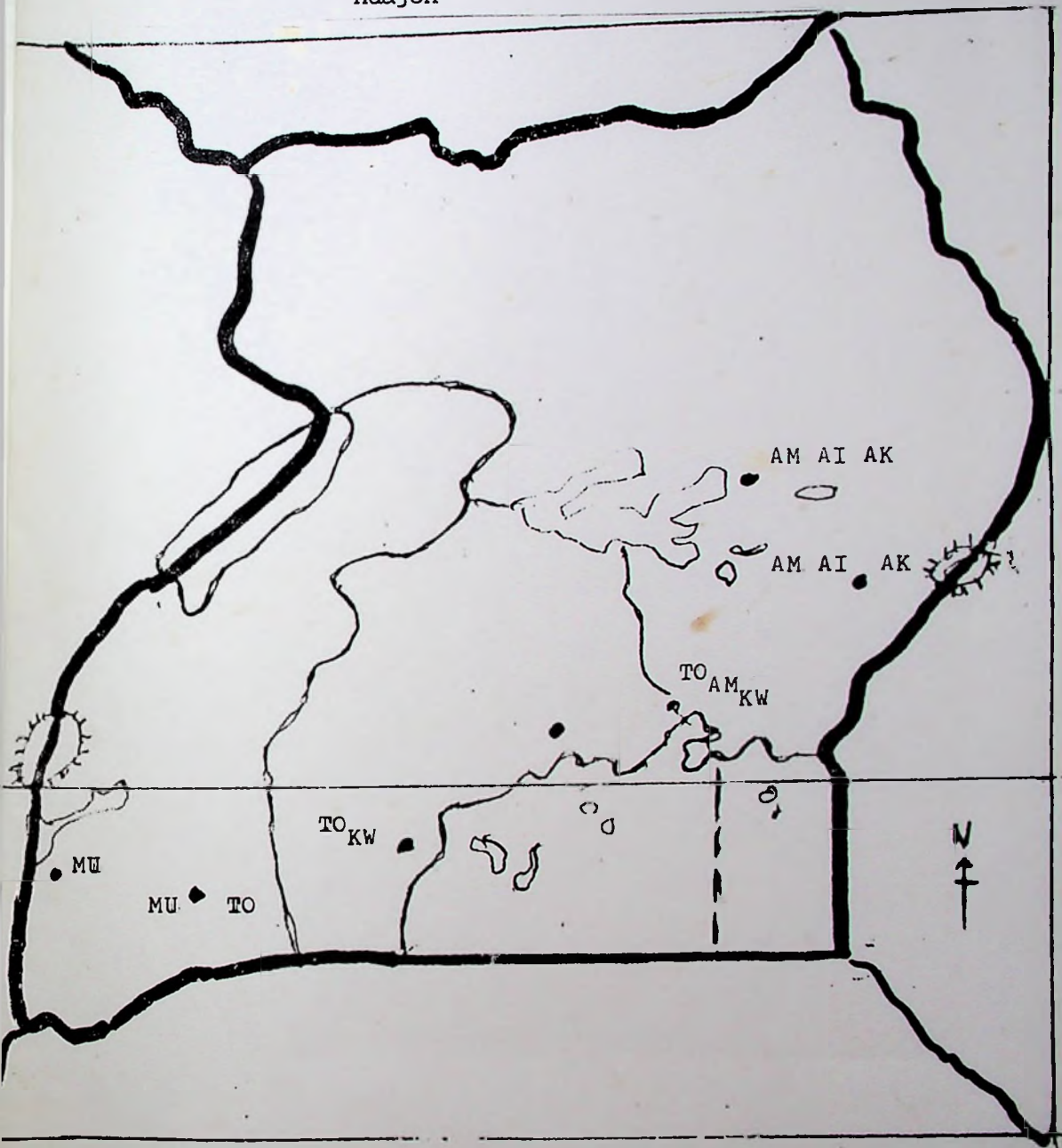
B= banana (Musa spp)
 P= pineapple (Ananas spp)
 M= mango (Mangifera spp)
 PA= passion fruit (Passiflora spp)
 PW= pawpaw (Carica spp)

Fig. 15 Distribution map for grains



SO= SORGHUM
MI= MILLET
MAI=Maize

Fig. 16 Distribution map for beverages, ainyeles and akiria
nuajon



TO = Tonto
 KW= Kwete
 MU= Muramba
 AM= Amarwa
 AI= Ainyeles
 AK= Akiria nuajon