MICROBIOTA AND MYCOTOXINS IN TRADITIONAL BEER OF THE GREATER KIMBERLEY AREA AND ASSOCIATED BREWING AND CONSUMPTION PRACTICES

by

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DECLARATION OF INDEPENDENT WORK

I, BRIDGET IKALAFENG, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree DOCTOR TECHNOLOGIAE: ENVIRONMENTAL HEALTH is my own work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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5/08/2008
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“Nothing can stop the man with the right mental attitude from achieving his goal; nothing can help a man with the wrong mental attitude.”

Thomas Jefferson

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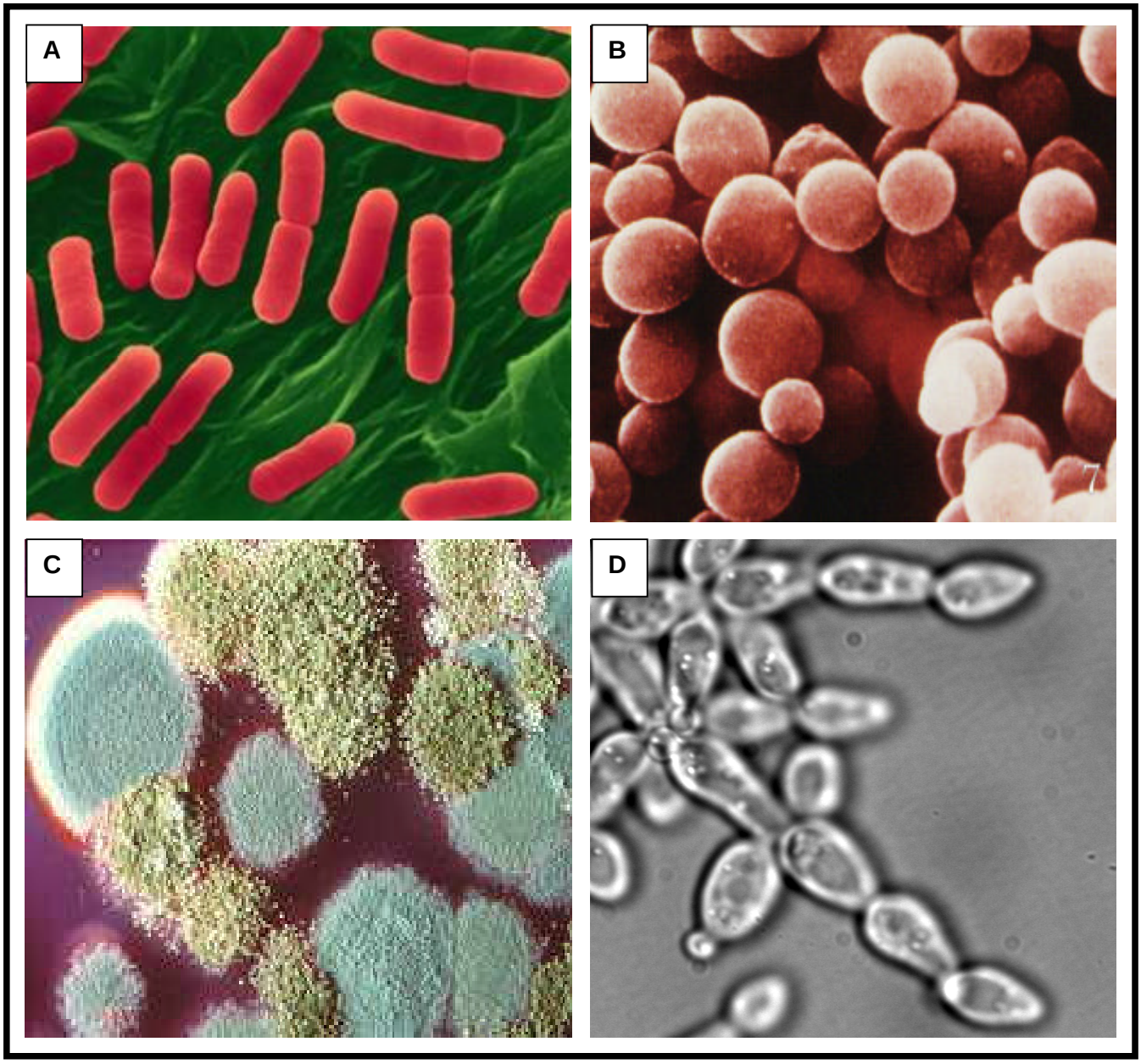
Figure i: Sampling of traditional beer



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SUMMARY

The purpose of this study was to evaluate brewing and consumption practices and to screen for micro-organisms and mycotoxins associated with traditional beer produced and consumed in the marginal urban settlements of the city of Kimberley in the Northern Cape Province of South Africa. The survey study revealed that traditional beer is no longer being brewed for traditional purposes only, as was the case in the past, but rather for commercial gain. Both brewers and consumers, however, appeared to be largely unaware of disease-causing micro-organisms present on the hands or bodies of handlers that can be transferred to the beverage during the handling process, and were seemingly not conversant with regard to the effects of hazardous ingredients sometimes incorporated during the brewing process. Unemployment and a lack of education emerged as pivotal factors related to the production of traditional beer and the ignorance of the associated safety thereof. The survey further indicated that although facilities such as the availability of potable water (taps in yards) and flushing toilets were sometimes in place, other facilities such as basins with hot running water were often not available.

In commercially produced and homebrewed traditional beer the mean counts for total coliforms and *Staphylococcus* spp. were *circa* 10^5 cfu.ml^{-1} whereas the TVC (Total Viable Counts) and total fungi counts were 10^6 and 10^7 cfu.ml^{-1} respectively. The total coliforms and *Staphylococcus* spp. counts for homebrewed traditional beer were approximately one log-phase higher than

the commercial version. The counts in the homebrewed beer probably originated from contamination during handling, while in the commercial product contamination originated either in the raw ingredients or during post-processing and consumption. Apart from staphylococci, considerable numbers of total coliforms indicating faecal contamination were noted. A rapid, easy, reliable and accurate technique that could be used to quantify the level of mycotoxins (deoxynivalenol and citrinin) in the beer was developed through validation of the ELISA Ridascreen methodology. Using this method, the deoxynivalenol (DON) level in the beer samples was found to exceed the recommended levels suggested by the European Union, while citrinin levels in the samples varied between 35.6 ppb and 942.2 ppb. In the case of citrinin there were statistically significant differences between spring, summer and winter samples, confirming the seasonal impact on fungal growth and consequent mycotoxin production. An R^2-value of 0.409 was noted between DON and citrinin, indicating a weak positive association.

Finally, an awareness programme in the format of a poster with accompanying subscripts was developed to address issues of safety and hygiene of traditional beer in the study area. The poster utilises animated-style colour images of selected practices that need to be addressed, accompanied by slogans summarising the particular image in English, Afrikaans and Setswana. It is envisaged that, as part of a comprehensive awareness programme, the poster will contribute greatly to the quality, safety and promotion of traditional beer in the area.

OPSOMMING

Die doel van die studie was om die brou-en verbruikspraktyke, sowel as die mikro-organismes en mikotoksiene geassosieër met tradisionele bier in marginaal-stedelike woongebiede aangrensend aan die stad Kimberley in die Noord Kaap, Suid Afrika te ondersoek. Die opname het getoon dat tradisionele bier nie meer vir tradisionele doeleindes gebrou word soos in die verlede nie, maar toenemend om kommersieële redes. Brouers en verbruikers het oorwegend onbewus voorgekom van siekte-veroorsaakende mikro-organismes wat vanaf die hande en liggame van hanteerders na die produk oorgedra kan word en blyk afsydig te wees ten opsigte van gevaarlike bestanddele wat soms tydens die brouproses bygevoeg word. Werkloosheid en ‘n verlies aan opleiding het geblyk belangrike faktore in die oningeligtheid rondom die veiligheid van tradisionele bier te wees. Die opname het verder getoon dat, hoewel lopende water (krane in erwe) sowel as spoeltoilette dikwels teenwoordig was, in ander gevalle fasiliteite soos wasbakke en warm lopende water nie beskikbaar was nie.

In kommersiële tradisionele bier was die gemiddelde totale-kolivorme en *Staphylococcus* spp. tellings *circa* 10^5 cfu.ml^{-1} terwyl TVC en totale fungi 10^6 en 10^7 cfu.ml^{-1} onderskeidelik was. Die totale-kolivorme en *Staphylococcus* spp. tellings vir tuisgemaakte tradisionele bier was ongeveer een log-fase hoër as die kommersieële weergawe. Die tellings in die tuisgemaakte produk het waarskynlik hul oorsprong in kontaminasie tydens hantering terwyl in die geval van die kommersiële bier kontaminasie waarskynlik vanaf die rou

bestanddele of tydens na-prossesering en verbruik afkomstig is. Behalwe vir staphylococci is beduidende getalle kolivorme, wat ‘n indikasie van fekale besmetting is, waargeneem. ‘n Vinnige, maklike, betroubare en akkurate tegniek vir die kwantifisering van mikotoksien-vlakke (deoxynivalenol en sitrinien) in bier is deur middel van validasie van die ELISA Ridascreen-tegniek ontwikkel. Deur die gebruik van hierdie metode is die deoxynivalenol vlakke in die bier bepaal en bevind dat dit die vlakke aanbeveel deur die Europese-Unie oorskry tewyl sitrinien vlakke tussen 35.6 dpb en 942.2 dpb gewissel het. In die geval van sitrinien is statisties-beduidende verskille tussen die lente, somer en winter monsters waargeneem wat die seisoenale invloed op fungus groei en uiteindelike mikotoksien produksie bevestig. ‘n R^2-waarde van 0.409 is tussen deoxynivalenol en sitrinien waargeneem, wat op ‘n swak positiewe assosiasie dui.

‘n Voorligtingsprogram in die formaat van a plakkaat met meegaande onderskrifte is onwikkel om die aangeleentheid van veiligheid en higiëne van tradisionele bier in die studie-area aan te spreek. Die plakkaat maak gebruik van geannimeerde kleur beelde van sekere praktyke wat aangespreek behoort te word, vergesel deur onderskrifte in Engels, Afrikaans en Setswana wat die spesifieke beeld opsom. Dit word voorsien dat, as deel van ‘n omvattende voorligtingsprogram die plakkaat tot die kwaliteit, veiligheid en bevordering van tradisionele bier sal bydra.

CHAPTER 1

INTRODUCTION

1.1 HISTORICAL BACKGROUND

The consumption of traditional beer by mostly black ethnic groups is as old as human development, although its use has only been documented since 1962. At that stage, traditional beer was only prepared for certain special occasions, such as traditional feasts, rituals, marriage ceremonies, funerals and cleansing ceremonies, coronations, harvest gatherings, cattle-slaughtering occasions, gatherings for the chopping of firewood, pre- and post-circumcision ceremonies, and births. Apart from its informal manufacturing, in South Africa traditional beer has been commercialised through the establishment of sizeable processing plants. Initially the government was a shareholder in a number of these companies, although these have since been taken over by local entrepreneurs with notable success.

In terms of its inherent characteristics, traditional beer is an alcoholic beverage produced and consumed in both rural and urban areas. It is a predominantly opaque and pinkish-brown drink with large quantities of suspended particles (Figure 1.1) and yeasts (Odumfa, 1985; Sibanda, Marovatsanga & Pestka, 1997). Although the beer generally exhibits the common denominator of being cereal based and the product of spontaneous lactic acid and alcoholic fermentation, its preparation differs from region to region. In some countries it is brewed with sorghum and millet as raw materials, while sprouted maize may also be used for production (Bvochora & Zvauya, 2001). Due to the beer's high nutritional value, relatively low alcohol

content (generally not exceeding 3%) and large quantities of suspended solids, some consumers use it as a staple food.

Figure 1.1: Pinkish, chocolate-brown traditional beer brewed in a 20-litre container

The handling of traditional beer is a highly ritualistic process. The beer may not be consumed whilst standing, nor should anybody be seated whilst holding a gourd of beer. The gourd must be continuously passed back and forth between individuals who belong to the same social category, with age and gender being the most important criteria. The drinking of traditional beer at ritual ceremonies binds together different groups or individuals and effects

reconciliation in times of difficulty (OIA, 1996). The beer is thus more than just a drink, because the whole social system is inextricably linked with this popular beverage. It is the essence of all festivities, the one incentive to labour, the first thought in dispensing hospitality, the favourite tribute of subjects to their chiefs, and almost the only votive offering in religious ceremonies. Nowadays, in most urban areas, strong national economic growth and other factors have resulted in the preparation of traditional beer as a means of generating income, and beer is no longer consumed for cultural purposes only. It is consumed for social reasons, is preferred mainly by people of low income because of its low cost, and is brewed predominantly at household level. Consumers advance various reasons for their consumption of the beer, such as to relieve stress or to "make life easier". In a sense, what was originally a traditional cultural beverage has now taken form as a psychological intervention.

Haggblade and Holzapfel (1989) describe the process of commercial brewing of traditional beer as malting, souring, boiling, mashing, straining, and alcoholic fermentation. Household brewing of traditional beer, as investigated in this study, involves the hand-mixing of 1400g brown bread, 1000g brown sugar and 10g wet yeast in a 20-litre container of lukewarm water, which is then left to ferment overnight. Household brewing of the beer is predominantly done by women, often with limited educational backgrounds, which is likely to be a factor in their disregard for the implementation and application of optimal hygiene measures. Neglecting such measures may

result in the presence of unacceptable numbers of micro-organisms and other hazardous substances in the beverage. The World Health Organisation (WHO, 1996) states that in order to reduce the occurrence of food-borne illnesses, it is crucial to understand the interaction of prevailing food safety beliefs, knowledge and practices.

1.2 PATHOGENIC AND OTHER MICRO-ORGANISMS IN TRADITIONAL BEER

Microbiota, whether undesirable or ubiquitous, have been shown to contaminate beverages via various routes. These include: 1) Unclean equipment, containers and surfaces; 2) Air (especially responsible for the introduction of "wild" species; 3) Inadequate storage facilities; 4) Improper handling of the ingredients by food handlers; and 5) Poor personal hygiene practices (such as scratching the hair, failing to cover infected wounds/cuts, failing to wash the hands after visiting the toilet, sneezing, coughing, and handling pets).

All of the above provide opportunities for microbiota contamination and growth, with the resulting formation of metabolites that can in turn cause flavours to go off and/or pose the risk of toxico-infection (Forsythe & Hayes, 1998). Members of the bacterial genera *Staphylococcus, Escherichia* and *Salmonella* are widely known to be associated with food-borne illnesses originating from traditional processes, and have been found throughout the developing world.

Staphylococcus aureus is widely described as the causative agent in many food-borne illnesses and is prevalent in the environment and on food handlers' hands (Desmarchelier, Higgs, Mills, Sullivan & Vanderlinde, 1999; Mosupye & Von Holy, 1999). *S. aureus* is a Gram-positive, facultative anaerobic coccus (1 µm diameter) and selected strains of this species cause food-borne illnesses. Its habitat is the skin and mucous membranes of warm-blooded mammals (Adams & Moss, 1997; Desmarchelier *et al.*, 1999; Martin & Myers, 1994). Toxins produced by *S. aureus* are relatively stable and can survive for up to 30 minutes in boiling water. General symptoms of toxin ingestion include nausea, vomiting, retching, abdominal cramps and prostration. Further symptoms, in more severe cases, include headache, muscle cramping and transient change in blood pressure and pulse rate (Bennett & Lancette, 1995).

Being a member of the *Enterobacteriaceae* family, *Escherichia coli* is a universal inhabitant of the gut of humans and other warm-blooded mammals, where it plays a major part in maintaining the normal physiological function of the intestines (Adams & Moss, 1997; Neill, Tarr, Taylor & Trafa, 1994). Therefore *E. coli* is often used as an indicator of faecal contamination and general hygiene (Gill, Friske, Tong & McGinnis, 1995). *E. coli* is a short, Gram-negative, non-spore-forming mesophilic rod capable of growing at temperatures of up to 50°C, with an optimum of 37°C (Adams & Moss, 1997). Until the late 1950s *E. coli* was claimed to be non-pathogenic; however some

E. coli strains were later found to be pathogenic (Garbutt, 1997; Vorster, Greebe & Nortje, 1994). *E. coli* strains are generally divided into the following groups: Enteropathogenic, enterotoxigenic, enteroinvasive, enterohaemorrhagic, and facultative enteropathogenic. These strains can cause acute gastroenteritis with the following symptoms: Abdominal pain, fever, vomiting, diarrhoea and bloody stools (Jacob, 1989).

Salmonella is another bacterial type implicated in traditional beverages, and has been reported in many countries as the agent most likely to cause illness (Bacon, Sofos, Belk, Hyatt & Smith, 2002). These organisms are small, motile, non-spore-forming, facultative, anaerobic, Gram-negative rods with a maximum growth temperature of 45 – 47°C , a minimum growth temperature of 5°C and an optimum growth temperature of 37°C (Mortimore & Wallace, 1994). Species included in the *Salmonella* genus are *S. typhi*, *S. enteritidis, S. typhimurium, S. heidelberg, S. derby and S. java* (Frazier & Westhoff, 1988). Food poisoning, known as salmonellosis, is commonly characterised by gastroenteritis and typhoid fever.

Although the three organisms mentioned are most often implicated in the microbial contamination of traditional beverages, organisms such as aerobic spore formers, other genera of the *Enterobacteriaceae* family and various yeasts and moulds have also been isolated. These micro-organisms interact with the beer ingredients during the brewing process, and may similarly produce an array of metabolites of which a number display toxic effects.

Because maize also supports fungal growth, the prevalence of mycotoxins and their associated fungal strains, introduced via especially the raw materials, transportation processes and storage practices, is generally regarded as a notable risk in the beverage-processing environment that should be carefully monitored.

1.3 MYCOTOXINS ASSOCIATED WITH TRADITIONAL BEER

Mycotoxins are produced as metabolites by filamentous fungi. Cereal-based products are especially prone to contamination by mycotoxins, which often results in waste and spoilage, especially in large-scale food storage and when foods are transported over long distances (FAO/WHO/UNICEP, 1987). Cereals particularly susceptible to contamination by mycotoxins include maize, barley, rye, oats and sorghum. The most prominent fungi associated with maize are *Fusarium moniliforme, Fusarium graminearum, Diplodia maydis* and *Aspergillus flavus* (Bullerman, 1997; Mostron & Raisbeck, 2007). Mycotoxins of importance in developing or Third World countries in Africa include deoxynivalenol, zearalenone, ochratoxin, fumonisin and citrinin.

These compounds may be highly reactive in humans and animals and are associated with acute diseases such as hepatotoxicity and carcinogenicity (Kuiper-Goodman, 1998). Mycotoxins can also be neurotoxic, cause organ pathology, affect the endocrine mechanism, or indirectly influence the immune system. Certain mycotoxins have been shown to either suppress or

stimulate the immune system, the latter leading to hypersensitive (allergic) reactions (Sharma, 1984, 1991). Many episodes of mycotoxin poisoning in livestock species have resulted in the death of animals.

1.3.1 Deoxynivalenol (DON)

Deoxynivalenol (Figure 1.2) is a mycotoxin belonging to the trichothecenes group, a most diverse group of mycotoxins known to be immunotoxic and potent inhibitors of protein synthesis (Prelusky, 1994). This group of mycotoxins is produced by a variety of *Fusarium* species, as well as a number of other fungal genera such as *Cephalosporium, Cylindrocarpon, Myrothecium, Trichoderma, Trichothecium, Stachybotrys* and *Verticimonosporium* (Smith, Lewis, Anderson & Solomons, 1994). They are in fact known to be produced by at least 24 different *Fusarium* species (Table 1.1) (Marasas, Nelson & Tousson, 1984). These mycotoxins are tricyclic sesuiterpenes with a basic 12, 13-epoxy-trichothecene-9-ene ring system. Trichothecenes are cytotoxic to mammalian cells and are known to cause toxic alimentary aleukia and fusariotoxicoses.

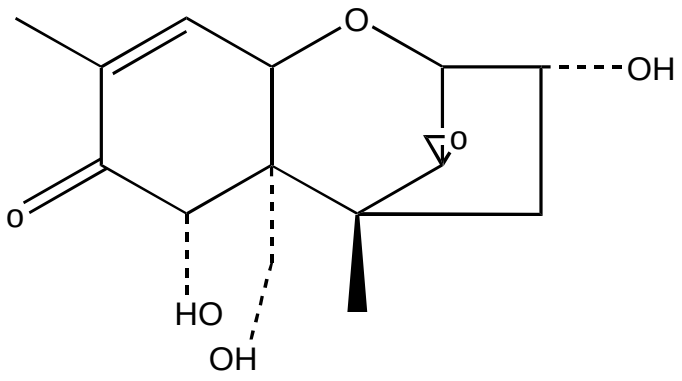

Figure 1.2: Schematic representation of deoxynivalenol (DON)

Table 1.1: Common trichothecene-producing *Fusarium* species (Bullerman, 1997; Mule, Logrieco, Stea & Bottalico, 1997; Pitt & Hocking, 1997; Smith *et al.,* 1994)

F. avenaceum	*F. poae*
F. acuminatum	*F. oxysporum*
F. camptoceras	*F. proliferatum*
F. chlamydosporium	*F. sambucinum*
F. compactum	*F. scirpi*
F. crookwellense	*F. semitectum*
F. culmorum	*F. solani*
F. equisiti	*F. sporotrichioides*
F. grameniarum	*F. subglutinans*
F. moniliforme	*F. tricinctum*
F. nivale	*F. tumidum*
F. nygamai	*F. venenatum*

Humans and other monogastric animals appear to display the greatest susceptibility to these toxins. DON is reported to often co-occur together with ochratoxin (Figure 1.3). Aflatoxins, ochratoxin A and fumonisins are other mycotoxins particularly implicated in human cancer (Battaglia, Hatzold & Kroes, 1996; Rheeder, Marasas, Thiel, Sydenham, Shephard & Van Schalkwyk, 1992; Wogan, 1991; Yoshizawa, Yamashita & Luo, 1994).

1.3.2 Citrinin

Citrinin (Figure 1.4) was first isolated from *Penicillium citrinum* prior to World War II (Hetherington & Raistrick, 1931). It has been identified in over one dozen species of *Penicillium* and several species of *Aspergillus,* for example

Aspergillus terreus and *Aspergillus niveus*. It has also been found in certain strains of *Penicillium camemberti* and *Aspergillus oryzae* in soy sauce (Manabe, 2001). More recently citrinin has been isolated from *Monascus rubber* and *Monascus purpureus*, which are industrial species used to produce red pigments (Blanc, Laussac, Le Bars, Le Bars, Loret, Pareilleux, Prome, Prome, Santerre & Goma, 1995), which would explain its detection in vegetarian food coloured with monascus. Citrinin is regularly associated with human foods such as corn, barley, wheat, oats, rye and rice (Abramson, Usleber & Marlbauer, 2001; Saito, Enomoto, Tatsuno & Uraguchi, 1971) and also with naturally fermented sausages (Anderson, 1995). Citrinin has been found to act as a nephrotoxin in a variety of animal species, with its toxicity varying among species (Carlton & Tuite, 1977).

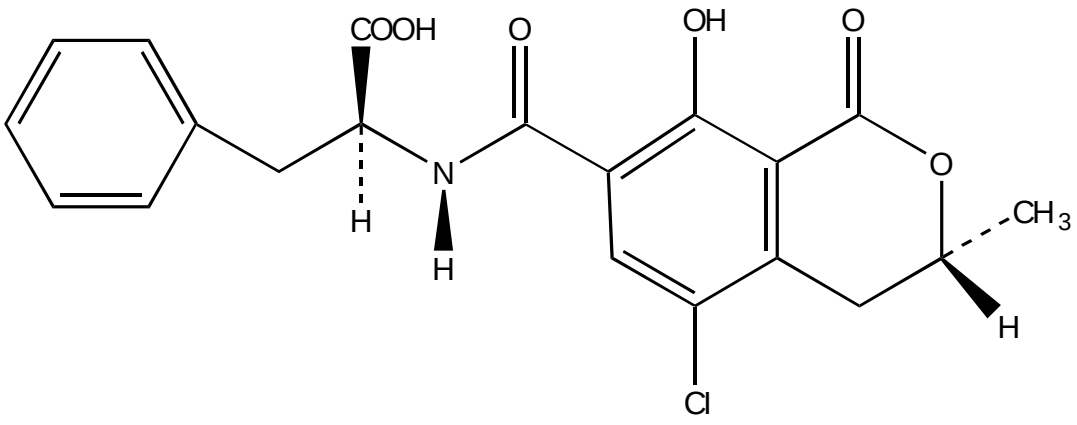

Figure 1.3: Schematic representation of ochratoxin

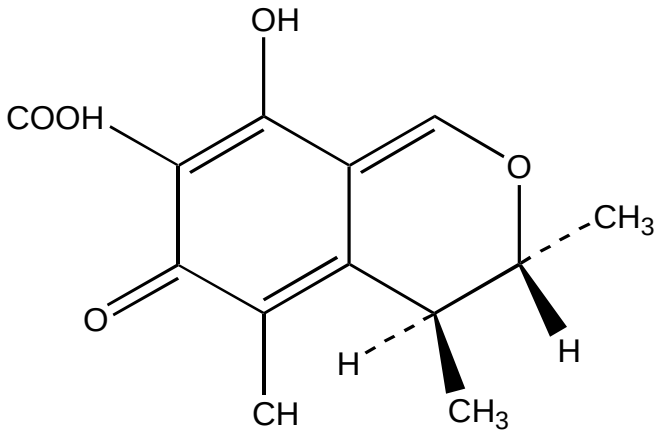

Figure 1.4: Schematic representation of citrinin

1.3.3 Factors influencing mycotoxin production

Fungal growth and the subsequent production of mycotoxins are often due to less stringent pre- and post-harvest treatment of crops. Apart from these, a number of practices associated with cereal production and household brewing may contribute to mycotoxin production. Such practices may include:

- Inadequate harvesting, storage and transportation processes;
- Lack of refrigerators;
- Inadequate fermentation equipment or facilities to prevent food contamination;
- Lack of sufficient knowledge of basic food safety measures on the part of brewers; and
- Inadequate control by regulative authorities.

Mycotoxin production may be further influenced by nutrient availability and environmental factors such as water activity and temperature (Bullerman &

Bianchini, 2007). *Fusarium graminearum*, for example, has an optimal growth range of 24 - 26°C and grows at a minimum water activity of 0.9. The effect of pH on fungal growth is temperature dependent, with a minimum growth pH of 2.4 at 30°C and 3.0 at 25°C and 37°C being reported (Wheeler, Hurdman & Pitt, 1991). *Fusarium sporotrichiodes*, which produces T-2 toxin, as well as deoxynivalinol, nivalenol and zearalenone, has an optimum growth temperature of between 22.5°C and 27.5°C and a minimum of -2°C. *Fusarium culmorum* is a psychotrophic strain and a known producer of deoxynivalenol. It is capable of growth at 0°C, with an optimum growth temperature of 21°C and a maximum of 31°C (Bottalico, Visconti & Lerario, 1982).

1.4 RATIONALE

The commercialisation and liberalisation of consumer goods has shifted the trend of the use and role of traditional beer into a major consumption industry. Inflation, unemployment and highly priced lager beers are all factors that have contributed to an increase in the commercialisation of indigenous traditional beer. The safe production of high-quality traditional beer depends, however, on the microbiological content of the raw materials used, hygienic conditions during production, and storage of the final product. Against this backdrop, this study was aimed at investigating selected microbiological and biochemical variables that would present a measure of the inherent hazards associated with the product. The information was supported by social surveys with regard to the handling of and exposure to the product

so as to give an indication of the risk associated with the consumption of traditional beer in the study area. This study was further motivated by a number of reports, including those by the South African Broadcasting Corporation (SABC, 2004) stating that certain brewers in the Northern Cape region add unacceptable toxic ingredients to their beer.

1.4.1 Aims

The aims of this study were to: 1) Assess the brewing practices utilised by the brewers and to elicit consumer perspectives with regard to traditional beer and the practices surrounding the brewing and consumption thereof; 2) Screen for selected micro-organisms in indigenous traditional beer with possible pathogenic features; and 3) Analyse the mycotoxins in the beer consumed in the study area.

By evaluating these parameters the study sought to establish recommendations towards improving the safety and quality of the product through proper monitoring and awareness programmes in the greater Kimberley area.

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CHAPTER 2

GENERAL HYGIENE AND BREWING PRACTICES OF TRADITIONAL BEER HANDLERS IN THE GREATER KIMBERLEY AREA

FOR SUBMISSION PARTIALLY OR IN FULL TO THE JOURNAL: ***JOURNAL OF ENVIRONMENTAL HEALTH***

2.1 ABSTRACT

The aim of the study was to assess the practices and knowledge regarding the safety and the brewing methods applicable to the manufacturing of traditional beer. A questionnaire survey was conducted among 30 brewers and 90 consumers, focusing on their personal hygiene and brewing practices. The data indicated that, while brewers were still using the same traditional brewing methods used in the past, the beer was now being brewed mainly for commercial purposes (75%) rather than for traditional reasons, as had previously been the case. Consumers indicated that they drank to relieve stress rather than for reasons of traditional belief. They were aware of certain toxic ingredients that were added to the beer, but were largely unconcerned about the possible health risks. Although the majority of the brewers did not have refrigeration facilities, improper hygiene practices did not appear to be the result of a lack of infrastructure, as facilities such as running water and flushing toilets were present in most households. Fifty-five percent (55%) of brewers reported that they washed the containers when dirty, while 45% washed them after each use. Unhygienic practices such as failure to cover the hair and wearing jewellery while brewing indicated a lack of knowledge regarding proper hygiene on the part of brewers, rather than deliberate negligence. The data gathered suggests that there is a need to establish and implement awareness programmes pertaining to personal and general hygiene.

This, together with regulations governing the licensing of township "shebeens" (informal canteens), should improve the general microbial quality of the beer and contribute to minimising health risks to the consumer.

Keywords: traditional beer, ingredients, hygiene, practices, knowledge

2.2 INTRODUCTION

In South Africa traditional beverages are known by a variety of names, depending on the part of the country in which they are produced and often also on the method of preparation. In the Kwazulu-Natal Province, for instance, traditional beer is known as “umqombothi”, while in the Free State Province it is called “mokoko” and in the Northern Cape Province “maiza”. The preparation of traditional beer predominantly requires the knowledge and expertise of the local people, mostly elderly women.

Traditional beer is normally prepared by mixing ingredients by hand in a twenty-litre container (drum), which is then covered tightly and left overnight to ferment. Fox and Cameron (1995) comment that this beer is consumed in large quantities in urban areas by low-income groups known to have little interest in the quality of food and drink or whether it may be detrimental to their health. In rural areas the consumption pattern is slightly different, as traditional beer is more often consumed as part of traditional ceremonies and/or rituals, such as weddings, funerals, and ceremonies to welcome back young men from initiation schools. In the past it was also part of the ceremony where groups of people gathered for communication purposes and contacted the spirits during their religious ceremonies (Rekdal, 1996). However, the use of traditional beer as a cultural symbol has diminished of late and much of its cultural value has been lost.

In terms of nutritional content, traditional beer provides the recommended daily requirement of thiamine and riboflavin (B vitamins) and 40% of the recommended daily requirement of niacin. However, many of these B vitamins are locked up in the yeast cells and cannot be digested unless the beer is boiled, which is seldom done (Lorri & Svanberg, 1995; OIA, 1996). The nutritional value aside, though, in recent times South African authorities have raised some concerns about the beer's microbiological composition, toxic substance content and alcohol content. Parts of the Northern Cape Province have particularly emerged as areas where alcohol consumption is on the increase. This increasing consumption is likely to encourage health-threatening diseases associated with both excessive drinking and the consumption of contaminated beverages. Since these concerns were illuminated by the South African Broadcasting Corporation, this trend appears to have intensified, not only in its effect on consumers but also by leading to indirect problems such as birth defects in infants (SABC, 2004). Awareness of the potentially hazardous nature of the beer as a result of unhygienic brewing practices has driven concerned authorities to physically dispose of homebrewed beer from "shebeens" in an attempt to discourage the consumption of beer that may be contaminated and unhealthy. However, such intervention by the police and Department of Health in the Northern Cape Province is only a short-term solution to the problem.

People will presumably continue to brew the beer regardless of actions taken by these authorities, unless the actual health hazards related to traditional

beer have been identified, scientifically investigated and documented. The aim of this study is therefore to cast light on the general brewing and hygiene practices of both brewers and consumers of traditional beer in the greater Kimberly area. These observations should aid the establishment of a conceptual and practical framework guiding the brewing and consumption of traditional beer in the Northern Cape Province.

2.3 MATERIALS AND METHODS

2.3.1 Questionnaire design

Validated questionnaires (Appendix 1) with structured closed and open-ended questions were administered to 30 brewers and 90 consumers in marginal urban settlements (Galeshewe, Phutanang, Vergenoeg, Number Two, Phomolong, Two Thousand, and Retswelele) surrounding the city of Kimberley. Structured interviews were conducted for the following reasons: 1) The interviewer was able to follow a well-defined structure, ensuring consistency in the interpretation of questions by all respondents; 2) The structure allowed control during the interview process and was designed to suit the level of the respondents; and 3) The interviewer was able to extrapolate on the interpretation of the questions where required (Czaja & Blair, 1996; Katzenellenbogen, Joubert & Abdool-Karim, 1997). The questions covered aspects of brewing hygiene, as well as personal and environmental hygiene. Specific questions focused on matters such as knowledge of ingredients, attitude towards beer consumption, and awareness in respect of

potential health problems that may result from the consumption of especially contaminated traditional beer.

2.3.2 Data collection

Prior to the interviews, arrangements were made with the brewers to secure their consent to gather information during the verbal interview sessions and to collect product samples. The stratified sampling method was used to select "shebeens" (informal canteens) in the greater Kimberly area, and three consumers were selected for every shebeen visited. The purpose of the interviews was explained to both brewers and consumers and a special effort was made to ensure that the respondents understood the purpose of the study.

Prior to assessment, the questions were also translated into the local languages, specifically Afrikaans and Setswana. The questionnaires were pre-coded and a code list was drawn up. In collaboration with the Department of Biostatistics of the University of the Free State, the questionnaires were analysed and the data presented in tables using frequencies, medians and percentiles.

2.4 RESULTS AND DISCUSSION

2.4.1 Profile of interviewees

The ages of the brewers ranged between 27 and 64 years, the majority of them (60%) being female. Twenty percent (20%) of the respondents had

not achieved a notable level of education, while 65% of the respondents (brewers) did have some form of education (grade 6 – 10). These profiles were similar to the findings of Umoh and Odoba (1999), namely that local beer brewers, especially women, are known to have attained relatively low levels of education. Most respondents had been in the brewing business for between 6 months and 15 years. The brewers furthermore indicated an income median of R880.00 per month. Seventy percent of the respondents revealed that beer brewing was their only source of income, while 30% depended on a spouse's income, pension and/or other small businesses such as street vending, taxi operations, gambling, or running a children's nursery (crèche).

2.4.2 Knowledge regarding brewing

The brewing of indigenous traditional beer involves malting, souring, boiling, mashing, straining, and alcoholic fermentation (Haggblade & Holzapfel, 1989). The method used by most brewers interviewed for this study (Table 2.1) was a normal basic recipe for preparing homebrewed beer as passed down by a family member, friend, or member of the community. This consisted of malt, usually the King Korn brand of "mthombo mmela" (maize), 1400g of brown bread, a 10g packet of wet yeast and a 1000g packet of brown sugar, all mixed in a twenty-litre container filled with lukewarm water. The beer is then covered tightly with a lid and left to ferment overnight. Mixing of the ingredients and overnight fermentation accomplishes the brewing process. During both the brewing and mixing process, the brewers use their hands to

mash the bread, while solid particles are removed the following day by sieving or straining through a cloth. After 48 hours the beer is usually ready for consumption. During the interviews the respondents expressed a different view from that of Haggblade and Holzapfel (1989) in that 80% were of the opinion that the fermentation period of 48 hours (2 days), which is recommended in the recipe, was unnecessary, and indicated a fermentation period of 24 hours to be sufficient for alcoholic fermentation (Table 2.1). The preference for a shorter fermentation period is advisable, as this is expected to limit microbial proliferation. Responding to a question on what they did with the leftover beer, 30% of the brewers admitted that beer left over from the previous day was sold to consumers the following day.

The majority of brewers reported that due to a lack of refrigerators, the beer was being stored at room temperature at the point of sale. Apart from the multiplication of the culture yeast, this practice is likely to increase the possibility of undesirable microbiological multiplication in the beer by wild yeasts and bacteria. The majority of brewers (75%) were brewing the "maiza" beer type in which maize is one of the ingredients, whereas 25% of the brewers were brewing "Barberton". None of the brewers interviewed were brewing the local beer "pine", which is normally brewed using traditional ingredients together with the addition of pineapple. Undocumented data indicates that Barberton is the type of beer normally mixed with unacceptable and/or hazardous ingredients such as battery acid, whereas maiza is normally purchased from breweries in 20-litre containers and resold to consumers.

Table 2.1: Respondent information regarding brewing practices

Variable	Percentage (%)
Source of knowledge regarding learned brewing method	
Mother	55
Friend	30
Neighbour	15
Fermentation period	
24 hours	80
48 hours	15
72 hours	5

2.4.3 Reasons for brewing

Although the majority of the brewers believed that the method they were using was traditional, the purposes for which they were brewing the beer differed. In the past traditional beer was brewed mainly for traditional occasions, such as the birth of a baby or the crowning of a king. Today, however, 75% of beer is brewed for commercial gain ("to make a living") and the remaining 25% for social/traditional occasions (Table 2.2). Traditionally, beer was stored in clay pots; however, all the brewers in this study were utilising 20-litre plastic drums and indicated that these were much easier to clean and did not rust, as opposed to metal drums that rust easily. The beer

is stored on the floor (Table 2.2) and is served in smaller 500ml plastic containers and not in a gourd as in the past (and as is still being done in certain parts of Africa).

2.4.4 Practices regarding the washing of hands and equipment during brewing (brewer data)

The human body is a reservoir for numerous micro-organisms, with the hands being the main agents for cross-contamination within food-handling establishments (Gordon-Davis, 1998). Legislation specifies that no person should be allowed to handle food if that person's hands have not been washed with soap and hot water (RSA, 1999). However, not all brewers commit to the procedure of washing hands, as stipulated in the proposed hygiene legislation. Fifty-five percent (55%) of brewers indicated that they washed their hands only when dirty, while 45% washed their hands only after visiting the toilet (Table 2.3). This practice of not washing hands is a concern, considering the fact that uncovered hands are used to mix the ingredients during brewing.

Table 2.2: Respondent reasons for selling beer

Variable	Percentage (%)
To promote tradition	25
To make a living	75
Storage	
On floor	100
Leftovers	
Discarded	50
Sold to customers	30
Other	20
Sediments	
Fed to animals	60
Reused	10
Discarded	30

Table 2.3: Measures used to evaluate hygiene practices to determine the possibility of contamination

Variable	Percentage (%)
Washing of hands	
When dirty	55
After using toilet	45
Washing of containers	
Before use	45
When dirty	55
Covering of hair	
In your opinion, is it necessary to cover the hair during brewing?	
Yes	85
No	15

Responses in respect of the washing of hands indicated that the respondents were unaware of bacteria such as *Staphylococcus aureus*, found on the hands of humans (Desmarchelier, Higgs, Mills, Sullivan & Vanderlinde, 1999). Desmarchelier *et al.* (1999) also emphasise that the use of hot running water and soap (preferably from a dispenser) is crucial for combating microbial pathogens during food processing.

With regard to the cleaning of containers, 55% of the brewers indicated that they washed the containers only when dirty and 45% reported that they washed the containers every time before use. Procedures were not necessarily the result of a lack of infrastructure, as the majority of the brewers had access to a water-tap about one metre from their households, as well as flushing toilet facilities.

2.4.5 Wearing of jewellery

Apart from being a physical hazard if ingested, jewellery is a potential reservoir for micro-organisms, because the skin under the jewellery provides a favourable habitat for proliferation (Trickett, 1998). Table 2.4 indicates that 75% of the respondents did not remove their jewellery when brewing. However, it may be assumed that the non-removal of jewellery was more a result of ignorance than negligence. One of the expected outcomes of this study is therefore a possible programme by means of which brewers and consumers are educated on the hazards of dipping their hands into the beer while wearing jewellery.

Table 2.4: Habits regarding the wearing of jewellery during brewing

Wearing of jewellery	Percentage (%)
Do you wear jewellery when brewing?	
Yes	75
No	15

2.4.6 Covering of hair

The covering of hair every time when food or beverages are handled is crucial to prevent loose hairs or dandruff from falling into the product. Hair is also a potential source of *S. aureus*. The covering of hair also discourages food handlers from running their fingers through their hair or scratching their scalp during brewing (NRAEF, 1992; Pelczar, Chan & Krieg, 1993). However, in the present study the majority of brewers disclosed that they did not cover their hair during the brewing or selling process (Table 2.3). To ensure quality production of the beer, the necessity of wearing aprons, covering hair, and keeping fingernails short / wearing gloves should be emphasised. The cloths and strainers used should be washed and disinfected regularly.

2.4.7 Practices regarding the reporting of illness (brewer data)

It is essential that brewers report any illnesses they may have. Sixty percent (60%) of the brewers indicated that they believed their beer to be safe. They argued that they usually tasted it themselves to ensure its safety, and they reported that should any illnesses be observed and associated with the

consumption of the beer, this was immediately reported to the resident clinic. The consumers, on the other hand, were negligent about the diseases and conditions such as headache, vomiting and, less frequently, seizures that may result from continued and/or heavy drinking of the beer. Trickett (1998) underlines the obligation of every food handler to report illnesses to a relevant authority.

2.4.8 Profile of interviewees (consumer data)

It was found that traditional beer was being mainly consumed by females (66%). Sixty-eight percent (68%) of all the consumers were single and 27% married, 4% widowed and 1% divorced, with ages ranging between 20 and 59 years. The levels of education ranged from grade 5 to grade 10 and only 20% of the consumers were employed. Forty-five percent (45%) of consumers had one or two dependants and 32% had three or four dependants (data not shown).

2.4.9 Knowledge regarding ingredients used for brewing (consumer data)

The consumers displayed varied knowledge of ingredients and some were ignorant about the content of the beverage. Fifty-one percent (51%) of the respondents reported that they were well informed about the ingredients used to brew the beer and were satisfied with the brewing procedure. They were also aware of the role of the added yeast. Forty-nine percent (49%), on the other hand, reported that they did not know what was used to brew the

beer, but that they were aware that yeast was added to allow fermentation and that they enjoyed the sour taste of the beer. This sour taste is due to the formation of organic acids during fermentation, as well as the presence of lactic acid bacteria, which produce acid during metabolic reactions to lower the pH to around 3.5 (Haggblade & Holzapfel, 1989).

2.4.10 Reasons for consuming traditional beer (consumer data)

Beer is a common means of exchange or payment for services rendered, and when it is available in abundance it is not only freely consumed but is often the principal or only food source for many people, sometimes for days on end. At many ritual ceremonial occasions, this beer plays a role in binding together different groups or individuals and sometimes effecting reconciliations in troubled times (OIA, 1996). Forty-two percent (42%) of the respondents indicated that they had been drinking the beer for more than 15 years, while 48% indicated that they were drinking it on a daily basis. A median of 300 litres of this beer were being consumed per week. Only 17% of the respondents reported on the variety of tastes that the beer might have, while 83% felt that it always tasted the same (data not shown). The consumers advanced reasons such as stress relief and making their lives easier as some of the factors influencing their drinking habits. It became evident that what was originally a traditionally cultural beverage has taken on a new form, that of a psychological intervention.

2.4.11 Practices regarding the reporting of illness (consumer data)

The consumers' responses illuminated the fact that they were paying little attention to the after-effects that may result from continued and/or heavy drinking of the beer. Ninety-three percent (93%) responded that they always ate food before drinking, although this was questionable, as the respondents appeared frail and weak, suggesting a lack of food or malnutrition. Only half the respondents indicated that they had recently visited a physician for a health check-up. Seventy-seven percent (77%) responded that they did not know anyone who had fallen ill from consuming this beer.

2.5 CONCLUSION

This study provides an overview of the general hygiene and brewing practices of traditional beer brewers and consumers in the greater Kimberley area of the Northern Cape Province. It is evident that brewers still make predominant use of indigenous recipes and brewing methods as used in the past. What is perturbing is the possibility of contamination resulting from the unhygienic practices of brewers. It should, however, be a priority to assist in the preservation of indigenous products, and the dignity inherent in their use, by educating communities about the harmful practices that could tarnish the traditional image of the beer.

There is a need for training and awareness in respect of personal and general hygiene practices, as it is clear that the brewing of the beverage is no longer a cultural practice, but has assumed the status of an economic enterprise,

and safety measures should be followed to safeguard the health of consumers and to maintain the integrity of brewers and their product. The necessity for the establishment of safety regulations is highlighted, as is the application of guidelines for the production of homebrewed beer. Such regulations should make a significant contribution towards sustainable development (nationally and for individuals) in line with the postulation that knowledge and value creation on the basis of indigenous knowledge is a form of mental and economic decolonisation (Williams & Muchena, 1991). The implementation of licensing for brewers willing to undergo training and to comply with food safety regulations should be investigated. This would entail ongoing supervision over personal and general hygiene practices. Monitoring of local brewers is necessary to ensure that they conform to the safety requirements.

As an additional outcome, the results of this study are to be presented to the Northern Cape Department of Health (DoH), and a partnership with the DoH will be proposed for the training of brewers in personal and business hygiene practices. Recommendations will also be made with regard to the application of sanitation methods that would limit the microbiological hazards associated with homebrewed beer. Routine monitoring and safety inspections by resident environmental health practitioners (EHPs) as part of the city's Municipal Health Services should be one of the main outcomes of such a partnership.

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APPENDIX 1

Microbiota and Mycotoxins in Traditional Beers of the Greater Kimberley Area and Associated Brewing and Consumption Practices

Homebrewed beer in

Area : _____ ☐ 1

Shebeen Number : _____ ☐ 2-3

Questionnaire

Mark answer/s with "x" in appropriate box.

Questionnaire number:

Section A:

1. Brewer data

Gender:
Male 1
Female 2

☐ 4

2. Age of home brewer

_____ years

☐☐ 5-6

3. Qualifications

None 1
Grade 1-5 2
Grade 6-10 3
Grade 11-12 4
Tertiary 5

☐ 7-8

4. Why do you brew this beer?

To make a living	1	9
To promote tradition	2	
Other reasons	3	

Specify _____

5. Do you brew for commercial purposes?

Yes	1	10
No	2	

6. If yes, how long have you been in this business?

_____ years	1	11-12
_____ months	2	

7. Is this the only income source for the household?

Yes	1	13-14
No	2	

8. If no, list other income sources.

Husband's income		15
Tuckshop	1	16
Pension	2	17
Other (specify)	3	18
	4	
	5	

9. How much profit do you make per week?

R_____ 19-22

10. What kind of beer do you prepare?

Pine	1	☐ 23
Maiza	2	
Barberton	3	
Both	4	
Other (Specify)	5	

_____

11. What ingredients do you use to prepare this beer?

_____ ☐ 24

_____ ☐ 25

_____ ☐ 26

_____ ☐ 27

_____ ☐ 28

_____

12. Are these the ingredients used traditionally?

Yes	1	☐ 29
No	2	

13. How do you prepare the beer?

_____ ☐ 30

_____

_____

_____

_____

14. How long do you leave the beer to ferment?

12 hours | 1
24 hours | 2
48 hours | 3

☐ 31

15. Where did you learn about this method?

_____ ☐ 32

16. Is this the traditional brewing method used?

Yes | 1
No | 2

☐ 33

17. What other brewing methods do you use?

_____

_____ ☐ 34

18. During which social occasions is this beer served?

Funerals	1		35
Weddings	2		36
Traditional rituals	3		37
Other (specify)	4		38

19. How often do you drink your own beer?

Always	1	39
Sometimes	2	
Never	3	

20. Do you know of people who have fallen ill from this beer?

Yes	1	40
No	2	

21. If yes, what were the symptoms?

Stomach cramps	1		41
Vomiting	2		42
Nausea	3		43
Other (specify)	4		44

22. What action do you take when your consumers fall ill as a result of drinking the beer?

_____ ☐ 45

23. What type of container do you use to brew the beer?

Plastic bucket	1	☐ 46
Metal bucket	2	☐ 47
Plastic drum	3	☐ 48
Other (specify)	4	☐ 49

24. Why do you prefer this container?

_____ ☐ 50

25. How many litres do you serve in a day?

_____ ☐☐☐ 51- 53

26. Do you refrigerate the beer?

Yes	1	
No	2	☐ 54

27. How long is the beer stored before it can be discarded as unfit for human consumption?

_____ ☐ 55

28. What do you use to strain the beer?

Metal wire strainer	1	☐	56
Cloth	2		
Grass strainer	3		
Other (specify)	4		

29. What do you do with the sediments?

Throw them away	1	☐	57
Use them again	2	☐	58
Mix with fresh ones for reuse	3	☐	59
Feed it to animals	4	☐	60
Other (specify)	5	☐	61

30. What do you do with the leftover beer?

Sell it again the next day	1	☐	62
Give it to the customers for free	2		
Throw it away	3		
Other (specify)	4		
Combination of 1&2	5		
Combination of 3&4	6		

31. Do you clean your containers?

Yes	1	☐	63
No	2		

32. If yes, when do you clean the containers?

Before preparing the beer	1	☐	64
When they are empty	2		
Once a week	3		

33. How often do you clean the containers?

_____ ☐ 65

34. When do you wash your hands?

After using the toilet	1	☐	66
Before using the toilet	2	☐	67
When they are dirty	3	☐	68
Other (specify)	4	☐	69

35. Why do you wash your hands?

_____ ☐ 70

36. Do you wear jewellery when brewing?

Yes	1	71
No	2	

37. Is it necessary to remove jewellery when brewing?

Yes	1	72
No	2	

38. Do you cover your head when brewing?

Yes	1	73
No	2	

39. Is it necessary to cover your head?

Yes	1	74
No	2	
Do not know	3	

40. Do you keep your fingernails short or long?

Short	1	75
Long	2	

41. Why do you keep them that length?

_____ 76

42. Do you have toilet facilities?

Yes 1
No 2

☐ 77

43. Do you usually wash your hands after visiting the toilet?

Yes 1
No 2

☐ 78

44. Generally, when is it most important to wash your hands?

_____

☐ 79

45. Is water always available to you?

Yes 1
No 2

☐ 80

46. How far is your water source from your shebeen?

_____ metres

_____ kilometres

☐☐☐☐ 81-84

47. Do you intend to stay in this business?

Yes 1
No 2

☐ 85

CONSUMER DATA

1. Gender

Male	1	☐ 1
Female	2	

2. Marital status

Single	1	☐ 2
Married	2	
Divorced	3	
Widowed	4	

3. Age

_____ ☐ 3

4. Qualifications

None	1	☐ 4
Grade 1-5	2	
Grade 6-10	3	
Grade 11-12	4	
Tertiary	5	

5. Employment status

Employed	1	☐ 5
Unemployed	2	

6. Number of dependants

1-2	1	☐ 6
3-5	2	
More than 5	3	

7. How long have you been drinking this beer?

1-5 years	1	☐ 7
6-10 years	2	
11-15 years	3	
More than 15 years	4	

8. How often do you drink this beer?

Every day	1	☐ 8
Once a week	2	
On weekends	3	
Twice a week	4	
Other	5	

9. Why do you drink it?

To quench thirst	1		9-12
For social reasons	2		
To get drunk	3		
Other reasons (specify)	4		

_____

10. Do you suffer from any of the following diseases?

Kidney failure	1		13
Diabetes	2		
High cholesterol	3		
Other (specify)	4		
	5		

11. Do you know of anyone who has ever fallen ill from drinking this beer?

Yes	1		14
No	2		

12. Have you ever been to a doctor?

Yes	1		15
No	2		

13. How does this beer usually taste?

A little sour	1	
Sweet	2	16
Other	3	

14. Does it always taste the same?

Yes	1	17
No	2	

15. Are you satisfied with the containers used to serve the beer?

Yes	1	18
No	2	

16. Do you know how this beer is prepared?

Yes	1	19
No	2	

17. Are you satisfied with the way in which it is prepared (by hand)?

Yes | 1
No | 2

☐ 20

18. Do you know why yeast is added?

Yes | 1
No | 2

☐ 21

19. Why do you choose traditional beer over modern beer?

_____

☐ 22

20. Do you eat before or after drinking beer?

Yes

No

21. What do you call this beer?

Maiza | 1
Pine | 2
Other | 3

☐ 23

CHAPTER 3

MICROBIOTA ASSOCIATED WITH HOMEBREWED AND COMMERCIALLY PRODUCED TRADITIONAL BEER IN MARGINAL URBAN SETTLEMENTS SURROUNDING THE CITY OF KIMBERLEY

FOR SUBMISSION PARTIALLY OR IN FULL TO THE JOURNAL: *FOOD CONTROL*

3.1 ABSTRACT

Due to the nature and origin of traditional beer, it is prone to spoilage by a variety of microbiota. In this study, samples of commercially produced traditional beer, as well as samples of indigenous homebrewed traditional beer, were collected from local brewers in the marginal urban settlements of Galeshewe, Vergenoeg, Number Two, Phomolong, Two Thousand and Retswelele in the greater Kimberley area of the Northern Cape Province. These samples were analysed for selected bacteria regarded as indicative of the quality and hygiene of the product. In commercially produced and homebrewed traditional beer the mean counts for total coliforms and *Staphylococcus* spp. were *circa* 10^5 cfu.ml^{-1} whereas the mean TVC and total fungi counts amounted to 10^6 and 10^7 cfu.ml^{-1} respectively. In terms of total coliforms and *Staphylococcus* spp., homebrewed traditional beer counts were about one log-phase higher than its commercial counterpart, whereas the counts for TVC and total fungi were both notably higher (*circa* 10^7 cfu.ml^{-1}). The counts in the homebrewed beer reflect considerable contamination by the handlers during production, whereas the higher-than-expected counts in the commercial version of the product suggest a notable degree of contamination either in the raw ingredients or during the post-processing and consumption stages. Further characterisation of staphylococci identified *Staphylococcus aureus, S. epidermidis, S. xylosus*, *S. homonis,* and *S. saprophyticus* to predominate in both types of beer. The mean pH for homebrewed traditional beer samples was 3.9 compared to 4.2 for commercially produced traditional beer, whereas the alcohol levels in both the homebrewed and commercially

produced traditional beer samples exceeded the alcohol content (4.5 %) set by the South African Department of Health for lager and related commercial beers. In addition to the prevalence of staphylococci, notable numbers of total coliforms indicated faecal contamination that may have resulted from poor sanitation, while the high counts of total fungi could be attributed mainly to yeasts associated with particular ingredients and the favourable pH. The implementation of sanitation guidelines and alcohol content standards for traditional beer, the licensing of home brewers, as well as training programmes in aspects such as good manufacturing practices (GMP) and proper personal hygiene, should be regarded as a serious priority in the study area.

Keywords: traditional beer, marginal-urban settlements, microbiota

3.2 INTRODUCTION

Traditional beer plays a major role in the diet of indigenous people of especially developing countries where it is used for a variety of purposes (Bvochora & Zvauya, 2001; Jespersen, 2003; Naumova, Korshunova, Jesperson & Naumov, 2003). The two types of traditional beer investigated in this study were firstly commercial traditional beer (maiza) and secondly traditional beer mixed and brewed entirely at home (umqombothi). The basic recipe for umqombothi includes malt, commercially-produced maize ("mthombo mmela"), brown bread, compressed yeast and brown sugar all mixed together with lukewarm water. The mixture is allowed to ferment overnight or longer. During the mixing process the brewers use their hands to mash the bread, and after fermentation the solid particles are removed by means of a sieve or a straining cloth. The end product varies from a pinkish to a brownish colour due to large quantities of suspended particles and yeasts (Odumfa, 1985). Maiza uses the same recipe as umqombothi with the difference that it is brewed and sold on a commercial basis. The commercial brewing of maiza involves malting, souring, boiling, mashing, straining, and alcoholic fermentation (Haggblade & Holzapfel, 1989); however, human intervention is limited as a result of the mechanical action inherent to commercial processing.

The production of good-quality, wholesome traditional beer requires a high standard of personal and general hygiene by all persons involved in the brewing process, mainly because the product provides a favourable

environment for the multiplication of microbiota. In addition, keeping the typical manufacturers and consumer base in mind, these individuals are often ignorant with regard to the quality of the product they consume. Studies have shown that lack of education on the part of food handlers has resulted in negligent practices, especially relating to sanitation and hygiene, during the production of indigenous products (Roy, Moktan & Sarkar, 2007). The practice of, for example, not washing the hands has been shown to result in up to 10^6 cfu.ml^{-1} growth of pathogenic organisms under the fingernails of food handlers (Abdussalam & Kaferstein, 1993; Mensah, Yeboah-Manu, Owusu-Darko & Ablordey, 2002). Martinez-Tome, Vera and Murcia (2000) highlight the education of food handlers as a crucial line of defence in preventing most types of food-borne illnesses. Organisms associated with food-borne illnesses relating to poor sanitation and improper food handling are particularly organisms such as coliforms and various species of the *Staphylococcus* genus (Lawrie, 1998; RSA, 1999).

The aim of this study was to determine the prevalence of selected micro-organisms in two different types of traditional beer produced in marginal urban settlements in the greater Kimberley area and to characterise the *Staphylococcus* spp. in particular, as they are commonly associated with poor hygiene. As species of the genus *Staphylococcus* are closely linked with specific sources of microbiota, their prevalence in the products were important when it came to shedding light on the aetiology of the contamination associated with traditional beverages. Comparing the two

beverages was necessary to ascertain the contribution of human intervention to the overall microbial load.

3.3 MATERIALS AND METHODS

3.3.1 Sampling protocol

To secure a representative sample, the stratified method was used to select thirty brewers of traditional beer from the following peri-urban settlements in the greater Kimberley area: Galeshewe, Vergenoeg, Number Two, Phomolong, Two Thousand, and Phutanang. Samples of both umqombothi and maiza were collected from these settlements over a period of one month during the summer season. The samples were aseptically collected between 09:00 and 14:00, in sterile sampling bags (Whirl-pack, NASCO), kept on ice to restrict microbial multiplication and transported to the laboratory for analysis. Serial dilutions were prepared in buffered peptone water (Biolab, SA) and 0.1ml of each dilution was plated on various selective media using the spread-plate method (Herbert, 1990).

3.3.2 Microbiological analysis

3.3.2.1 Total viable counts (TVC)

Enumeration of total viable counts was done on plate count agar (PCA) (Merck, SA) after incubation at 25°C for 72 hours (Houghtby, Maturin & Koenig, 1993; Vorster, Greebe & Nortje, 1994).

3.3.2.2 Total coliforms

Enumeration of total coliforms was done on Chromocult coliform agar (Merck, SA) after incubation at 37°C for 48 hours. Colonies were dark-red to purple in appearance.

3.3.2.3 *Staphylococcus* spp.

Baird Parker agar (Biolab, SA) was used for the isolation of *Staphylococcus* spp. and the plates were incubated at 35°C for 48 hours. Grey-black colonies with a clear zone around the colony were regarded as *S. aureus*. Identification was confirmed with the rapid latex agglutination test (Slidex Staph Plus test kit, Bio Merieux, France) (Personne, Bes, Vandenesch, Brun & Etienne, 1997; Van Griethuysen, Bes, Etienne, Zbinden & Klutymans, 2001). *Staphylococcus* colonies isolated from Baird Parker agar, which were not classified as *S. aureus,* were plated on blood agar and incubated for 24 hours at 35°C. These strains were identified using the API-Staph system (Nagase, Shimizu, Kawano, Yamashita, Yoshimura, Ishimara & Kawamo, 2002) and APILAB software in accordance with the manufacturer's specifications (Bio Merieux, France).

3.3.2.4 Total fungi (yeasts and moulds)

Detection and enumeration of total fungi were done on potato dextrose agar (PDA) acidified to pH 3.5 with tartaric acid (Merck, SA) (Christen, Davidson, McAllister & Roth, 1993; Frank, Christen & Bullerman, 1993). Plates were incubated for 72 hours at 25°C.

3.3.3 Alcohol and pH analysis

The pH was measured with a calibrated food-grade pH meter (Hanna Instruments) while the alcohol content was measured with a refractometer (Cole-Parmer, Model EW-81010-18, Swiss-Labs, SA).

3.3.4 Statistical analysis

Data were recorded as the means of at least triplicate analyses. In the case of comparative experiments $P \leq 0.05$, using the t-test was regarded as significant.

3.4 RESULTS AND DISCUSSION

3.4.1 Total viable counts (TVC)

Measuring total viable counts (TVC) is a convenient tool in assessing the general microbial contamination of foodstuffs. The mean TVC for maiza samples were around 10^5 cfu.ml^{-1} (Figure 3.1Aa) and for umqombothi up to 10^7 cfu.ml^{-1} (Figure 3.1Ba). These high counts could be the result of contaminated ingredients being used, contamination during or after preparation (mixing) of ingredients, or inadequate storage practices. In assessing the TVC, however, a basal medium and not a selective medium was used; thus the contribution of the yeast and mould population to this count should be kept in mind. The 2-log-phase lower TVC counts in commercially produced traditional beer are likely to be due to the fact that brewing such beer involves more mechanical action and less human activity.

3.4.2 Total coliforms

Members of the total coliforms are widely distributed in the intestines of humans and warm-blooded mammals and are also the predominant facultative anaerobes in the bowel (Collins, Lyne & Grange, 1995; Hayes, Ralyea, Murphy, Carey, Scarlett & Boor, 2001). In addition to the fact that a number of species in this group are pathogens and the causative agents of food-related illnesses, they are regarded as indicators of faecal contamination. In the beer these organisms thus provide an estimate of faecal contamination in either the raw materials or during production or consumption (Eisel, Linton & Muriana, 1997; Strech & Southgate, 1991).

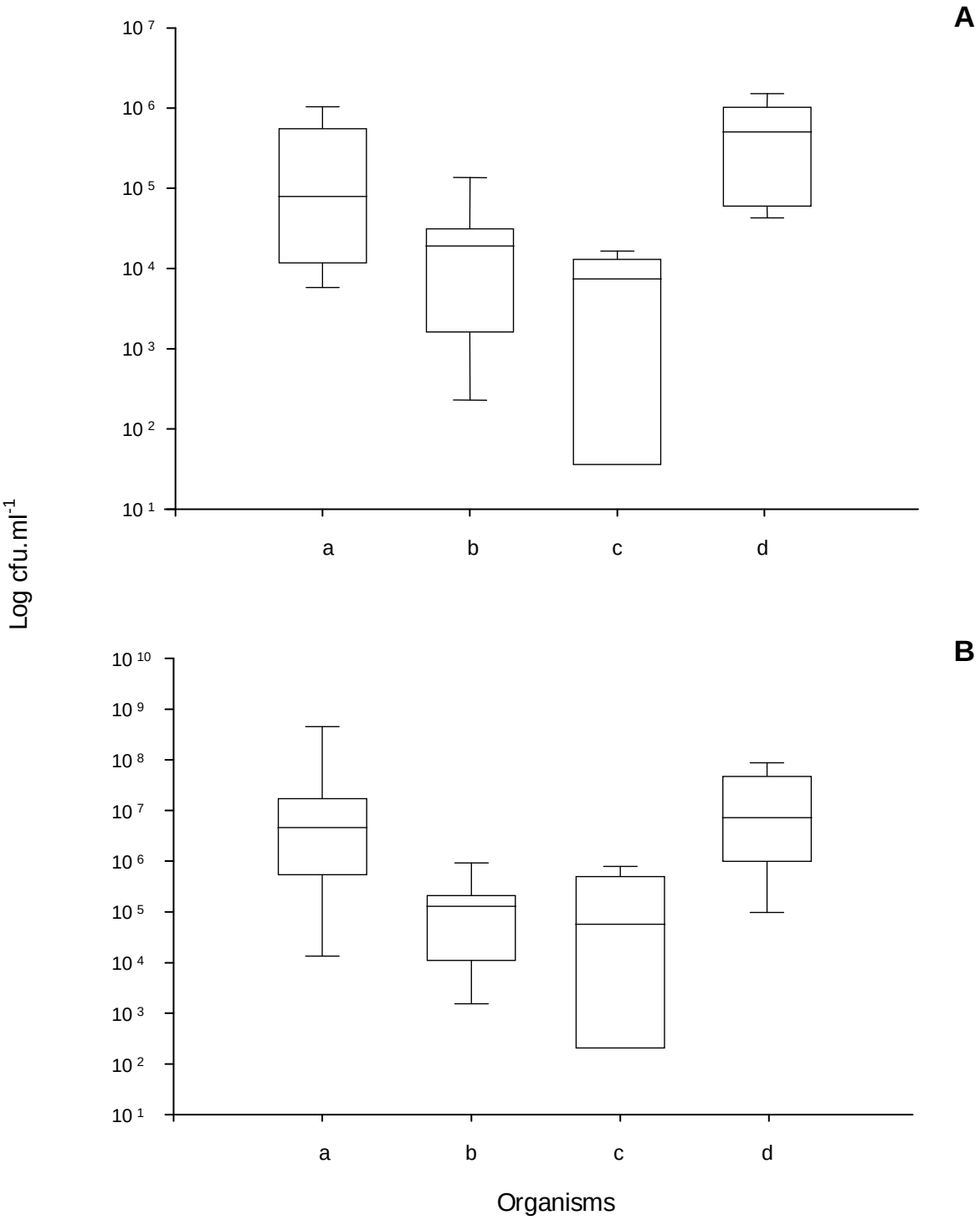

Figure 3.1: Microbial distribution in commercially produced and homebrewed traditional beer

(A) Commercially produced traditional beer (maiza): (a) TVC, (b) total coliforms, (c) *Staphylococcus* spp. and (d) total fungi; (B) Homebrewed traditional beer (umqombothi): (a) TVC, (b) total coliforms, (c) *Staphylococcus* spp. and (d) total fungi

Total coliforms were isolated from 80% of the commercially produced beer samples with counts in the region of 10^4 cfu.ml^{-1} (Figure 3.1Ac) and 100% of the homebrewed beer samples with counts ranging from 10^5 to 10^6 cfu.ml^{-1} (Figure 3.1Bc). Roy *et al.* (2007) reported coliform counts in 92% of samples taken from traditional fermented foods in India. When keeping in mind that the organism *Escherichia coli* dominates the total coliform population, it is likely that the legislative guideline of 10 cfu.ml^{-1} would be exceeded in both products investigated in the present study, as would the infective dose level of 10^5 cfu.ml^{-1} stipulated by the South African Department of Health (RSA: DoH, 2000) for foodstuffs sold to the general public. The samples could have been contaminated by various routes such as poor production processes or poor handling by the brewers.

3.4.3 *Staphylococcus* spp.

Staphylococcus spp. is commonly found on the hands, under the fingernails and in the nose, throat and mouth of healthy people. When present in those areas of the body these bacteria do not cause any harm and people may be unaware that these bacteria can cause illness due to the toxins that they produce (Trickett, 1998). The organism itself may cause spoilage and produce off-flavours, but food poisoning is normally associated with the production of heat-labile enterotoxins (Anderson, Rönner & Granum, 1995; Eley, 1992; Hittu and Punj, 1999; Reed, 1994).

For maiza the counts of staphylococci in traditional beer were *circa* 10^4 cfu.ml^{-1} and for umqombothi 10^5 cfu.ml^{-1} (Figure 3Ac and 3 Bc). These exceeded the maximum limit stipulated in legislation (10^2 cfu. ml^{-1}) and – in the case of umqomothi – the infective dose levels for foodstuffs (10^5 cfu. ml^{-1}) (RSA: DoH, 2000). Although high numbers of the above-mentioned organisms were expected in umqombothi, the fact that they also occurred in the commercial version was notable. Since samples were collected from domestic environments, the counts in the latter are likely to be the result of poor handling (improper storage, unclean containers and/or communal sharing of the drink) rather than any processing activities.

3.4.3.1 Classification and aetiology of *Staphylococcus* spp.

Maiza samples were contaminated with *S. aureus* (38%), *S. epidermidis* (18%), *S. xylosis* (1.2%), *S. hominis* (0.8%), *S. capitis* (22%) and *S. saprophyticus* (0.2%). *Staphylococcus* species identified in umqombothi were *S. aureus* (56%), *S. epidermidis* (30%). *S. hominis* (0.4%), *S. capitis* (16%) and *S. saprophyticus* (0.4%) (Table 3.1). In terms of *S. aureus* the levels were to be expected, as human skin has been reported to be the most common source of *S. aureus*, from which point the organism finds its way into the air and onto clothing from where it may further contaminate foods. The relative prevalence of this species in umqombothi was notable; however, a number of other staphylococci also produce staphylococcal enterotoxins (SEs) and are thus capable of causing food-borne illnesses. Kumar, Baboota, Agarwal, Javed and Alka (2008) report *S. epidermidis* to be a common pus-

forming microbe that is responsible for the development of various forms of acne vulgaris. *S. xylosus,* which was isolated from both homebrewed and commercially produced traditional beer, is a common habitant on the skin of domestic and wild animals and is predominantly associated with nasal dermatitis or "sore nose" (Nagase *et al.*, 2002). *S. xylosus* has also been isolated from the teat skin of cows, as well as from the nares of humans (Nagase *et al.*, 2002). As is the case with the majority of other *Staphylococcus* species, *S. capitis* (present in 22% of maiza isolates and 16% of umqombothi isolates), is commonly associated with the skin of humans and the hides of warm-blooded animals (Euzéby, 2003). The presence of *S. saprophyticus*, has in particular been linked to poor sanitary hygiene, as it is frequently associated with acute urinary tract infections.

Table 3.1: *Staphylococcus* species in traditional beer

Organism	Umqombothi	Maiza
S. aureus	38%	56%
S. epidermidis	18%	30%
S. xylosus	1.2%	0%
S. homonis	0.8%	0.4%
S. capitis	22%	16%
S. saprophyticus	0.2%	0.4%

3.4.4 Total fungi

Yeasts and moulds play both a positive and a negative role in fermented products. The positive role of yeasts lies in their contribution to the fermentation process in especially bread, alcoholic beverages and other products. However, on the negative side, they may also act as spoilage organisms. The high yeast counts (10^6 to 10^7 cfu.ml^{-1}) for both homebrewed and commercially produced beer (Figure 3.1Ad and Figure 3.1Bd) were to be expected, as yeasts were included in the ingredients. However, since the umqombothi-brewing process makes use of an "open" fermentation process, it was to be expected that numerous wild fungi would contaminate and multiply in the product. Due to the predominance of the culture yeasts, however, it is unlikely that wild yeasts and moulds will reach such high numbers in the product after a 24-48-hour fermentation period as to have a notable effect in terms of sensory quality and safety. Contamination of the product by yeasts other than as part of the ingredients used for fermentation has, however, been shown to produce off-flavours.

3.5 COMPLIANCE AND SAFETY

In this study the majority of samples of both commercially based and homebrewed beer suggest contamination close to or above the infective doses, raising concerns about consumers' health. The higher microbial counts found in homebrewed traditional beer in comparison with commercially produced traditional beer led to the conclusion that homebrewed beer may

present a marginally higher risk. The presence of total coliforms, as well as particular species of staphylococci, suggests a degree of ignorance amongst home-based brewers in respect of the fundamental aspects of proper hygiene and safe housekeeping in addition to a lack of basic infrastructure. It is advisable that protocols regarding brewing be established and brought into effect by local authorities, concomitant to coherent strategies for the licensing of informal breweries. Such strategies should serve as regulatory measures for controlling hygiene and quality. In order to assist local brewers in brewing beer of sound quality, the following recommendations are made: 1) Brewers should be educated regarding quality control and possible microbial hazards that might be present in the beer; 2) Local municipalities, via environmental health practitioners, should regularly monitor the brewers in terms of proper hygiene, sanitation, management of waste, and refrigeration; 3) The negative health effects of alcohol should be highlighted and the consumer's right to enquire about the ingredients used for brewing should be stipulated; 4) The benefits of a healthy diet should be emphasised; 5) Entrepreneurship through proper business management should be encouraged; 6) Licensing of brewers should be implemented and properly monitored; and 7) National standards and guidelines should be drafted for microbial chemical parameters in particularly homebrewed beer.

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CHAPTER 4

PREVALENCE OF MYCOTOXINS IN SOUTH AFRICAN TRADITIONAL BEER I: QUANTITATIVE ANALYSIS OF DEOXYNIVALENOL

FOR SUBMISSION PARTIALLY OR IN FULL TO THE JOURNAL: *FOOD COMPOSITION AND ANALYSIS*

4.1 ABSTRACT

Cereals used in the production of beer have been shown to often be contaminated by various fungi able to produce mycotoxins such as deoxynivalenol (DON), citrinin, fumonisins and zearalenone. The aim of this study was to determine whether one such mycotoxin, deoxynivalenol (DON), is present in traditionally brewed beer and whether its presence and concentration is influenced by seasonal changes. Beer samples were collected from households in informal and marginal urban settlements in the Kimberley area of the Northern Cape Province during the summer, winter and spring seasons. The DON concentrations were determined using an ELISA method that was standardised and validated for DON detection in traditional beer. Several parameters were varied in order to optimise the quantitative analysis. The recovery level for DON was 97% when a zero ppb DON standard was used as diluent and 103% when deionised H_20 was used as diluent. Standard curves of data from traditional beer samples of known mycotoxin concentrations were identical to those prepared with data from the kit standards. The intra-assay and inter-assay variations for the analytical method (Ridascreen ELISA DON) was 9% and 13.6% respectively. The mean DON concentration during the summer was 13.6 ppb, while in spring it was 7.3 ppb and during winter 6.2 ppb. A statistically significant difference ($p \leq 0.01$) was found when comparing the DON levels of the summer samples with the other two seasons respectively. The data indicates that parameters such as temperature and humidity may have an effect on the DON concentration of the traditional beer. Further investigation is required to determine the risks of

contamination with DON, while strategies should be developed to ensure that the beer produced does not pose a health risk to consumers.

Keywords: traditional beer, deoxynivalenol, temperature, humidity

4.2 INTRODUCTION

Several studies have reported on the occurrence of mycotoxins in traditional beer produced from malt, as well as several other cereal-based commodities (Jajic, Juric & Abramovic, 2007; Njapau & Mzungaile, 1993; Tanaka, Hasegawa, Yamamoto, Lee, Sugiura & Ueno, 1998). The maize used in the brewing process has frequently been reported to be contaminated with fungi (Sydenham, Shepard, Thiel, Marasa, Shepard, Van Schalkwyk & Kock, 1990). Apart from maize, mycotoxin-producing fungi have been reported to also infect barley, wheat, corn and sorghum (Larsen, Hunt, Perrin & Ruckenbauer, 2004). One of the mycotoxins, deoxynivalenol (DON), occurs in a variety of plant products, particularly cereals contaminated by *Fusarium graminearum* (Edwards, 2004; Maiorano, Blandino, Reyneri & Vanara, 2007). Other mycotoxins found in cereal products are ochratoxin, fumonisin, citrinin, nivalenol and zearalenone. DON is, however, part of the B-trichothecene group of toxins, and ingestion of large amounts of this toxin in a short period of time has been indicated to cause acute toxicity, while small doses over a prolonged length of time often result in chronic ailments.

Apart from being a particularly highly toxic mycotoxin producing a wide range of immunological and other disturbances in humans, it is especially noted for inducing feed refusal in addition to immunosuppressive effects in animals (CAST, 1999) and has been shown to be a major problem for pig farmers, as it can cause pathologies such as food refusal and emesis amongst pigs (Sydenham *et al.*, 1990). In humans the immunosuppressive effects of DON

may have a concerted effect with other immune disorders such as HIV, which is highly prevalent in sub-Saharan Africa. Chronic low-dose intoxication due to DON is characterised by symptoms such as anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunological effects (Rotter, Prelusky & Pestka, 1996). In South Africa DON has been reported to play a major role in the aetiology of oesophageal cancer in the former Transkei region (Sydenham *et al.,* 1990).

In South African rural settlements and poorer urban areas, some of the brewers use their own sorghum, malt and/or maize for brewing, although most use commercially available products. The majority of these brewers do not have proper facilities, causing the raw products used for beer production to be stored for prolonged periods under unfavourable extrinsic conditions. These predominantly include humidity and temperature levels conducive for the proliferation of fungi. At the time of the study, South African legislative limits had only been established for aflatoxin and patulin (Odhav & Naicker, 2002) and not for any of the other mycotoxins. Due to the potential health impacts of DON, legal limits for DON have been set for a number of products in several countries, and levels have also been applied by the Grain Institute of the South African Agricultural Research Commission (ARC). Some of these limits are portrayed in Table 4.1.

Table 4.1: Summary of legal maximum levels of DON in various human food and animal feed products

Product	Limit	Reference
Human food	500 – 1000 ppb	ARC Grain Institute (2001)
Animal feed	1000 – 10000 ppb	ARC Grain Institute (2001)
Infant food, bread and noodles	2 ppb	ARC Grain Institute (2001)
Whole-wheat flour, bran and pasta	750 ppb	CAC (Codex Alimentarius Commission) FAO/WHO (2003)

The Ridascreen DON test (AECI Amersham, SA) has been effectively used for the detection of DON in cereals and feed, and works both rapidly and with accuracy. This is a competitive enzyme-linked immunoassay intended for the quantitative detection of DON in cereal grains and other commodities, including animal feed. The Ridascreen method has, however, not been applied or optimised for traditional beer. Methods currently used for mycotoxin analysis in beer are mainly based on high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) (Gilbert & Anklam, 2002). These methods are, however, time consuming and require complex extraction protocols, the purification of samples, expensive laboratory equipment, and highly trained

personnel. In contrast ELISA methods are rapid, sensitive and accurate, with the added benefit of being relatively inexpensive and simple. However, immunology-based methods such as ELISA have not yet been validated at sufficiently low levels and are limited to the range of matrices examined to date. The aim of this study was thus to validate an ELISA method (Ridascreen DON) (AECI Amersham, SA) to determine the DON concentrations in traditionally brewed beer and to determine the influence of temperature and humidity on DON concentrations over a given sampling period.

4.3 MATERIALS AND METHODS

4.3.1 Sampling protocol

Samples were collected in marginal urban settlements in the Kimberley area of the Northern Cape Province of South Africa, including Galeshewe, Vergenoeg, Number Two, Phomolong, Two Thousand, and Phutanang. Samples were collected in the winter, summer and spring seasons. The climatic data for the sampling period was obtained from the regional weather office at the Kimberley airport. Thirty brewers of indigenous South African beer were selected from the study area using the stratified method, and samples were collected during the early hours of the morning from freshly produced drink (both homebrewed and commercially produced traditional beer). Samples were aseptically collected in sterile sampling bags (Whirl-pack, NASCO), transported on ice to the laboratory, and centrifuged for 5 minutes (1000g) to separate the supernatants from the cells, with the latter being aliquoted and distributed onto 96-microtitre plates.

4.3.2 Quantification of toxins in beer samples

A Ridascreen DON ELISA kit (AECI Amersham, SA) was used to detect the presence of DON in the traditional beer. Fifty (50) µl of the sample or standard, 50 µl detecting antibody and 50 µl antigen-peroxidase conjugate were added to antibody-coated microtitre plates, agitated and incubated for 30 minutes at room temperature. The plates were washed with 230 µl per well of kit-wash solution and dried. This was followed by the addition of 100 µl peroxidase substrate to each well. The enzymatic reaction was allowed to proceed for 20 minutes at room temperature in the dark and terminated by the addition of kit-stopping solution. The plates were read on a plate spectrophotometer at a wavelength of 450nm and absorbance values were expressed as a percentage of the 0 ppd DON standard. A standard curve was constructed by plotting percentage absorbance against DON concentrations, and the concentrations of the samples were read from the standard curve.

4.3.3 Validation of Ridascreen ELISA

The Ridascreen ELISA for DON was further used to determine the inter- and intra-assay variation by conducting assays in triplicate on the 6 samples with the highest known DON concentrations, and using the mean and standard deviations to calculate the variations. To evaluate the influence of H_20 (used for the preparation of the beer and also as a diluent for beer samples), samples were diluted with deionised H_20 or with the 0 ppb standard from the kit. The DON concentration was determined for both the undiluted and the

diluted samples, and standard curves were constructed for the beer dilution series as mentioned above. The samples containing the highest DON levels were selected for validation purposes and assayed as undiluted.

4.3.4 Statistical analysis

Data were recorded as the means of at least triplicate analyses. In the case of comparative experiments $P \leq 0.05$, using the t-test was regarded as significant.

4.4 RESULTS AND DISCUSSION

The level of DON in the undiluted sample was 41.3 ± 2.0 ppb (Table 4.2). This sample was diluted by 50% in the 0 ppb DON standard, resulting in a DON value of 20.0 ± 3.0 ppb, while when diluted in H_20 the sample had a value of 21.3 ± 0.4 ppb. The DON recovery for the sample diluted in the 0 ppb standard was thus 96.9 ± 14.6 %, while for the sample diluted in H_20 the concentration was 103.3 ± 2.0%. This indicates that either of these diluents can be used in the Ridascreen DON ELISA. The results obtained for various diluted samples were plotted on the same graph as the kit standards (Figure 4.1) and show that the curves obtained using diluted beer samples were similar to those obtained using the kit standards. The evaluation of inter- and intra-assay variability is shown in Table 4.3. The relative standard deviations obtained for samples varied between 16.3% and 3.9%. The data further shows that for samples with a DON concentration less than 20 ppb the intra-assay variation was below 10%, while it was 16.3% for samples with a DON concentration higher than 20 ppb. Plate-to-plate variations were 13.6 %. The

atmospheric data shown in Table 4.4 substantiates the significant statistical difference of DON concentration levels between the summer and winter/spring seasons respectively. The DON levels in summer, when humidity and temperatures are high, were significantly higher than in the other two seasons (Table 4.5). The presence of DON in the samples analysed concurs with previous studies that have shown high incidences of DON in European and Canadian beer samples (Scott, Kanhere & Weber, 1993; Schwartz, Casper & Beattie, 1995) and confirms that DON is a stable molecule and able to survive the brewing process. Fifty-seven percent (57%) of the samples collected in the summer season had a DON concentration ≤11 ppb, while the remaining samples (43%) had DON concentrations ≥11 ppb (Table 4. 5). The DON concentration in summer ranged from 4.7 to 31.3 ppb with a mean concentration of DON in these samples of 13.6 ppb.

Table 4.2: Effect of sample diluent on DON recovery using the Ridascreen ELISA

Sample	Diluent	DON (ppb)	DON recovery (%)
Neat	N.A.	41.3 ± 2.0	N.A.
Diluted 1/2	0 ppb standard	20.0 ± 3.0	96.9 ± 14.
Diluted 1/2	H_20	21.3 ± 0.4	103.3 ± 2.0

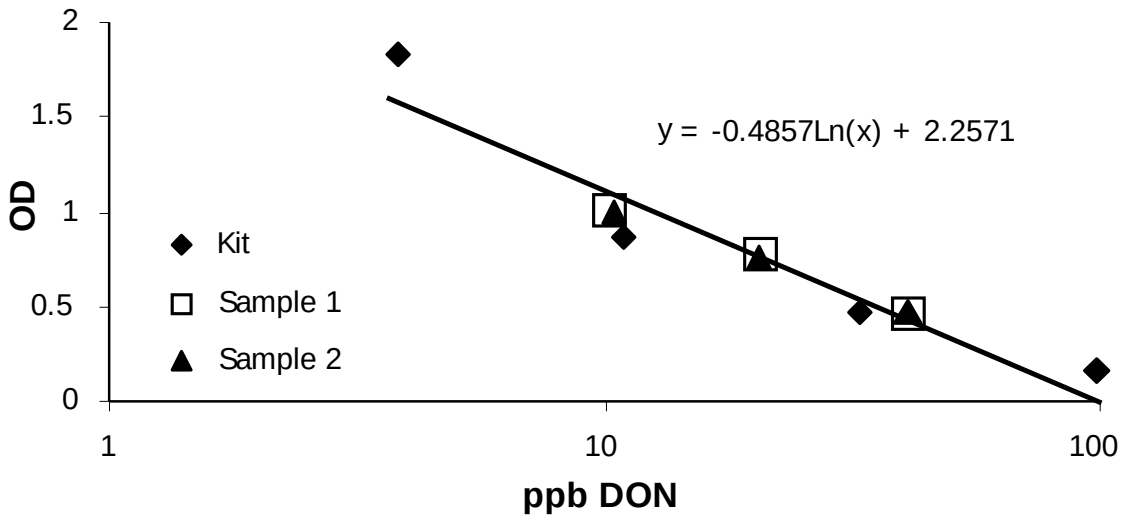

Figure 4.1: Comparison between the DON kit standard curve and the standard curve using dilution series of indigenous traditional beer

(Linear – no transformation)

Table 4.3: Inter-assay variation for DON concentrations of beer samples using the Ridascreen ELISA

Sample ID	Mean	Standard deviation	% Standard deviation
2	32.1	1.8	5.7
3	26.7	1.0	3.9
10	10.0	1.6	16.3
13	28.0	2.6	9.4
58	37.9	1.4	3.7
63	20.0	4.4	15.2
		Mean % standard deviation	9.0

Fusarium is the source of production of fumonisin and trichothecenes. Methods capable of detecting DON concentrations ≤5 ppb show that up to 50% of the samples may be contaminated when the appropriate species of *Fusarium* infects growing cereals. The DON levels of traditionally brewed South African beer samples collected during the summer season were high compared to the levels (2 ppb) detected by Schwartz *et. al.* (1995) who furthermore reported that 80 – 93% of the DON toxin is carried over in the final beer sample. The majority of the spring and winter samples measured ≤11 ppb DON (Table 4.5). The mean for the spring samples was 6.2 ppb DON and the samples ranged from 4.1 ppb to 8.3 ppb, whereas the mean for the winter samples was 7.3 (ranging between 1.7 ppb and 32.0 ppb).

DON concentrations in the summer samples differed significantly from the spring ($P \leq 0.001$) and winter ($P \leq 0.01$) sample sets. There was, however, no significant difference in DON concentrations between the spring and winter sample sets ($P = 0.4$). A probable explanation for the seasonal differences in DON concentration could be the major variations in atmospheric conditions experienced in the study area and the influence thereof on mould growth. Schrödter (2004) confirms that specific extrinsic parameters such as high temperatures and humidity stimulate the growth of fungi with resulting increased toxin production.

Table 4.4: Environmental conditions in Kimberley, Northern Cape Province of South Africa, during the sampling months

Date collected	Season	Temperature (°C)	Humidity (%)	Rainfall (mm)
June 2003	Winter	16.3	6.5	0
January 2004	Summer	37.1	29	0
August 2004	Spring	19.4	7.1	0

Table 4.5: Summary of DON levels in traditional beer samples collected in the Kimberley district during selected spring, summer and winter periods

	Samples assayed for DON			DON ppb		
Season	**Total**	**≤11 ppb**	**> 11 ppb**	**Lowest**	**Highest**	**Average**
Spring	31	94%	6%	3.1	12.1	6.2 ± 2.1
Summer	14	57%	43%	4.7	31.3	13.6± 8.6
Winter	25	88%	12%	1.7	32.0	7.3 ± 6.5

It was to be expected that the DON level in summer, when humidity and temperatures are higher, would be higher compared to the other two seasons. The slight differences in temperature and humidity between the winter and spring samples did not contribute to significant differences in DON concentration.

In summary, this study demonstrates that the Ridascreen DON ELISA is a rapid (≤1 hour), sensitive and accurate method for successful analysis and detection of DON and possibly other mycotoxins in traditional beer. Although gas chromatography has often been recommended for the detection of mycotoxins and is reported to be sensitive and accurate with the advantage of detecting other related compounds, its disadvantage, however, is that DON is non-volatile and must be derivatised to form a stable derivative suitable for analysis. The GC methods are also expensive and require skilled operators. In contrast, the Ridascreen DON ELISA may be used as a less expensive, more robust alternative for the successful and accurate quantification of large numbers of samples for mycotoxins.

The study further indicates that environmental and/or atmospheric conditions, such as temperature and humidity, have a definite effect on DON levels. This highlights the need to assess levels of various mycotoxins in products produced from cereals on a seasonal basis. In terms of health risk, the DON levels in a large number (43%) of the summer samples tested were above the EU recommended safety levels (2 ppb). This may pose a risk to the consumers, keeping in mind the consumption levels and socio-economic profile of typical consumers. Although ochratoxin and DON do not necessarily coexist in traditional beer, the fact that these mycotoxins can be present as co-contaminants further highlights the problems associated with the ingestion of multiple toxins that have an effect on the health of consumers.

It is recommended that South African legislative bodies consider DON limits for traditional beer similar to the European Union limits for commercial beer. The maize used for brewing should also be monitored for contaminants to prevent batches containing harmful toxins from being sold to the public. Since the multiplication of moulds has been linked to improper storage conditions, proper controls should be put in place to avoid or limit mycotoxin formation during storage, especially for areas of South Africa known for extreme temperature and humidity conditions.

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CHAPTER 5

PREVALENCE OF MYCOTOXINS IN SOUTH AFRICAN TRADITIONAL BEER II: SEASONAL VARIATION OF CITRININ

FOR SUBMISSION PARTIALLY OR IN FULL TO THE JOURNAL: *FOOD ADDITIVES AND CONTAMINANTS*

5.1 ABSTRACT

A seasonal study on citrinin concentrations in traditional beer was conducted using a standardised enzyme-linked immunosorbent assay. Seventy samples of indigenous South African beer were collected during the hot, mild and cold seasons from marginal urban settlements in the Kimberley area of the Northern Cape Province of South Africa. The respective mean temperature and humidity readings were 37°C and 29% for summer, 19.4°C and 7.1% for spring, and 16.3°C and 6.5% for winter. The samples were analysed for the mycotoxin citrinin using a commercial Ridascreen (citrinin) ELISA kit, validated for purposes of the study. The recovery level achieved for citrinin in beer samples using the kit was 117, ±15%. The inter-assay and intra-assay variations were 17.8% and 14.8% respectively. Standard curves prepared using traditional beer samples were identical to the standard curves prepared using the kit standards. The mean citrinin concentrations found in the various samples were 227.6ppb, 99.0ppb and 185.7ppb for the summer, spring and winter seasons respectively and the levels ranged between 35.6 ppb (min) and 924.2 ppb (max). Statistically significant differences were found between the citrinin levels of the summer and spring samples ($p \leq 0.05$) and also between the citrinin levels of the spring and winter samples ($p \leq 0.001$). The notable levels of citrinin in some of the samples can, with prolonged exposure, have an adverse impact on the health of consumers, and further studies into the origin and levels of this mycotoxin should be a priority.

Keywords: citrinin, traditional beer, temperature, humidity

5.2 INTRODUCTION

Although the detection of citrinin has been reported in foods by several authors, its presence in particularly fermented products has been reported as a potential threat to public health (Benett & Klich, 2003; Liu, Wu, Su, Chung & Yu, 2005). Citrinin is a fungal metabolite and a common food contaminant, which can cause the deterioration of liver or kidney function in animals (Chan & Shiao, 2007; Sweeney & Dobson, 1998). Concon (1988) found that food contaminated with *Penicillium citrinin* causes functional and morphological renal damage and results in increased urination. Ingestion of these mycotoxins by humans occurs mainly through plant-based foods, but also as residues and metabolites present in animal-derived foods.

Citrinin is produced by moulds such as *Aspergillus* and *Penicillium* and the species most notorious for producing this mycotoxin are *P. citrinin* and *P. expansion,* although *P. verrucosum* has also been shown to produce citrinin (El-Banna, Pitt & Leistner, 1987; Gupta, 2007). *Penicillium citrinin* infects grains such as wheat, oats, barley, rye and rice and also beverages such as beer (Abramson, Usleber & Marlbauer, 2001). Fungal species that produce citrinin are predominantly mesophilic and grow at a temperature range of 5°C to 40°C with an optimum of between 26°C and 30°C (ICMSF, 1996). Pitt (1993) reports citrinin to be produced at temperatures ranging from 15°C to 37°C. Environmental factors such as temperature, humidity and rainfall, which differ from season to season, also play a role in fungal growth, which may result in mycotoxin production.

Citrinin levels varying from 0.28 to 6.29 µg/g in *Monascus* fermented products have been reported by Liu *et al.* (2005). In countries such as Denmark samples of barley and oats have been reported to be contaminated with 160 – 20000 $\mu g.kg^{-1}$ of citrinin. The aim of this study was to validate the Ridascreen citrinin ELISA assay for screening indigenous South African beer for the occurrence of citrinin, as well as to investigate possible seasonal variations of citrinin concentrations in traditional beer, brewed in the greater Kimberley area.

5.3 MATERIALS AND METHODS

5.3.1 Sampling protocol

Triplicate analyses were done throughout, with significance level selected at $P \leq 0.05$. Seventy beer samples were collected from various traditional beer brewers during spring (August, 2003), summer (January, 2004) and winter (June, 2004) in the greater Kimberley area of the Northern Cape Province of South Africa. Brewers of indigenous South African beer were selected using the stratified method, and the samples were collected in the early hours of the morning from freshly produced supplies using sterile sampling bags (Whirl-pack, NASCO). The samples were transported on ice to the laboratory and stored at –20°C. Samples were consequently homogenised and centrifuged for 5 minutes at 1000 x g to separate the supernatants from the cells. The supernatants were aliquoted and stored in 96-well trays.

5.3.2 Quantification of citrinin in beer samples using ELISA

Ridascreen citrinin ELISA kits (AECI Amersham, SA) were used to analyse the mycotoxins, and the manufacturer's protocol was followed. The sample or standard (50 µl per well) and 50 µl per well of anti-citrinin antibody were added to citrinin-coated plates. The mixture was agitated and incubated for 10 minutes at room temperature, and at the end of the incubation period the plate was washed four times with 250 µl per well of deionised H_20. The plate was dried and 100 µl of the secondary antibody conjugate solution was added to the wells. The reaction was allowed to proceed for 10 minutes at room temperature and the unreacted antibodies were removed by washing three times with 250 µl of deionised H_20 per well. Two drops of either substrate or chromogen were added to each well and mixed. The plate was incubated in the dark for 5 minutes at room temperature, after which the reaction was stopped by the addition of a stopping solution. The plate was read on a plate spectrophotometer at 450 nm and the percentage absorbance was calculated for all the wells using the 0 ppd citrinin standard as the 100% reference. The kit standards were used for the construction of a standard curve obtained by plotting percentage absorbance against citrinin concentrations. The consequent values were read from this standard curve.

5.3.3 Validation of the Ridascreen ELISA for citrinin

The inter- and intra-assay variation of the Ridascreen ELISA for citrinin was determined to establish whether assays were reproducible. Samples were assayed in triplicate on three occasions. The mean and standard deviations

for the samples were calculated and the results were then used to calculate inter- and intra-assay variations.

5.4 RESULTS AND DISCUSSION

5.4.1 Validation of Ridascreen ELISA (citrinin) for traditional beer

The citrinin recovery level was 117, ±15%, and the inter- and intra-assay variations were 17.9% and 14.9% respectively (Table 5.1). Figure 5.1 shows the results obtained using kit standard and beer standard. The curve obtained using beer samples is parallel to the curve obtained using the standards provided with the kit.

5.4.2 Screening of traditional beer for citrinin using Ridascreen ELISA kits

The Ridascreen citrinin assay has proven to effectively analyse a large number of beer samples compared to the HPLC, TLC and GC methods, which are currently the most commonly used methods for detecting a wide variety of mycotoxins. Citrinin was detected in all the samples, but in varying concentrations during the different seasons. The mean citrinin concentrations found in the various samples were 99.0 ppb, 227.6 ppb an,d 185.7 ppb for the spring, summer, and winter seasons respectively and the levels ranged between 35.6 ppb (min) and 924.2 ppb (max). Citrinin concentrations during spring and summer were found to differ significantly ($P \leq 0.05$). This was also the case between the spring and winter ($P \leq 001$) sample sets (Figure 5.2). A summary of the citrinin concentrations is shown in Figure 5.2 where the mean,

highest and lowest concentrations, as well as the standard deviations, are depicted.

Table 5.1: Citrinin recovery

(The beer was spiked with a known citrinin concentration and the spiked sample was assayed using the Ridascreen citrinin ELISA)

Inter-assay variation	17.9%
Intra-assay variation	14.9%
% spike recovery	117.1% ± 15.3%

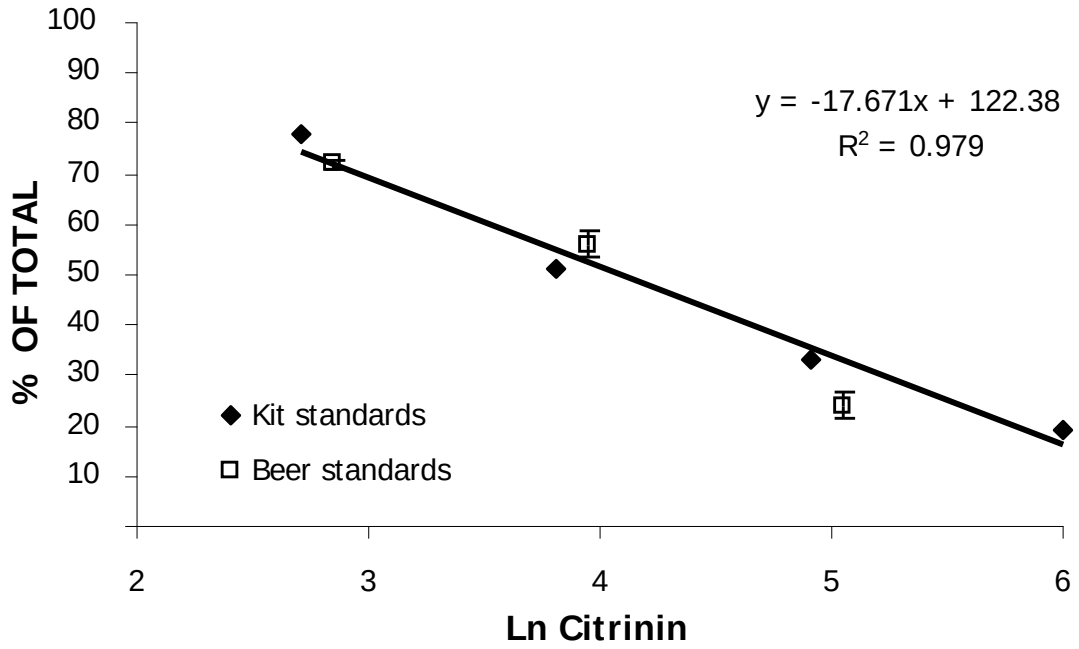

Figure 5.1: Comparison between the citrinin kit standard curve and the standard curve using dilution series of indigenous traditional beer

(Ln transformation)

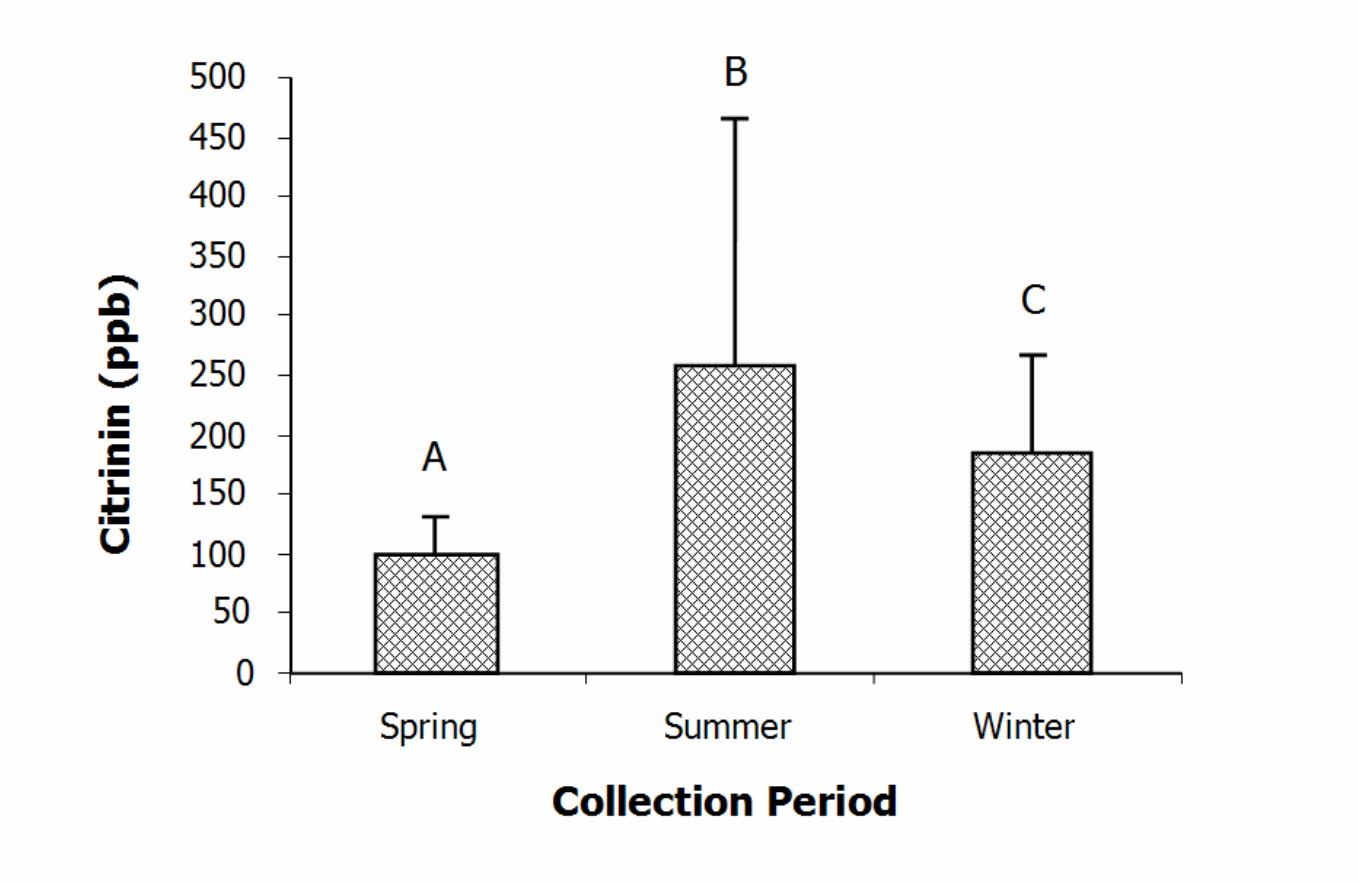

Figure 5.2: Comparison of citrinin concentrations (mean) in samples collected in different seasons of the year

(A/B significant $P \leq 0.05$; A/C significant $P \leq 0.001$)

The results obtained for this study differed from those obtained in a previous study on South African beer, which reported that citrinin was not detectable in the beer, as it was destroyed during the mashing step (Odhav & Naicker, 2002). The levels of citrinin in the present study were likely determined by the climatic conditions, as well as the more sensitive Ridascreen method used for detection. Due to the differences in temperature and humidity between the different collection periods, it was to be expected that there would have been differences between the citrinin levels of the samples collected at these

intervals. The citrinin levels in the samples collected during summer when the humidity and temperature are high, were found to be significantly higher than during the other seasons. The growth of fungi is known to be enhanced by high temperatures and humidity (Schrödter, 2004) and the findings of this study thus substantiate reports that countries with warmer climates are prone to mycotoxin-contaminated grains and consequently to beers containing toxins (Okoye, 1987; Okoye & Ekpenyong, 1984).

The fact that citrinin was detected at high levels indicates that mycotoxins are not destroyed during the mashing step in the production of the products surveyed in this study. The levels of citrinin detected in the beer could pose serious health risks to consumers, especially keeping in mind that these substances accumulate in the liver over lengthy periods of time.

Habitual consumers of these beverages therefore have greater exposure to the harmful effects of mycotoxins. Prevention and/or control in the areas where traditional beer is brewed and consumed in large quantities is thus of the essence.

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CHAPTER 6

GENERAL CONCLUSIONS

6.1 INTRODUCTION

The purpose of this study was to evaluate brewing practices and to screen for micro-organisms and mycotoxins associated with traditional beer produced and consumed in the marginal urban settlements of the city of Kimberley in the Northern Cape Province of South Africa. In terms of empirical work, Chapter 2 reports on the general hygiene and manufacturing practices of traditional beer handlers, as very little work has been done in this area. Chapter 3 reports on the microbial population in both traditionally and commercially produced beer, as this relates to the handling practices and presents a measure of the level of hygiene. Chapter 4 reports on the mycotoxin deoxynivalenol, which is known to be associated with, amongst other things, oesophageal cancer in humans, while Chapter 5 describes the presence and seasonal variations of another mycotoxin, citrinin, which has nephrotoxic properties and is a causal determinant in kidney disease.

6.2 SUMMATIVE REMARKS REGARDING THE KNOWLEDGE AND PRACTICES OF TRADITIONAL BEER HANDLERS (CHAPTER 2)

Knowledge regarding the brewing methods and hygiene practices of traditional beer brewers, and the consumers' awareness of potentially hazardous ingredients that may be used for brewing traditional beer in the region concerned, were assessed by means of a questionnaire survey. The study revealed that traditional brewing techniques (and ingredients) were

being used by all the brewers interviewed. It further emanated that beer was no longer being brewed for traditional purposes only, as had been the case in the past, but rather for commercial gain. Brewing for commercial purposes was often being done in an unhygienic manner and brewers were largely unaware of disease-causing micro-organisms present on their hands or bodies that can be transferred to the beverage during the handling process.

Unemployment and a lack of education emerged as pivotal factors related to the production of traditional beer and the ignorance in respect of the safety thereof. It was further noted that consumers were seemingly ignorant about the effects of hazardous ingredients sometimes included during the brewing process, and this is one of the many reasons why preventative measures should be implemented by the relevant authorities. The survey study further indicated that although facilities such as the availability of potable water (taps in yards) and flushing toilets were in place, other facilities such as basins with hot running water were not available. The lack of such facilities poses concerns as to the production of safe and uncontaminated beer. If these problems are not addressed, microbiologically contaminated traditional beverages will continue to be served and may significantly contribute to the alarming statistics of diarrhoea and other infectious diseases.

6.3 SUMMATIVE REMARKS REGARDING THE MICROBIOLOGICAL SURVEY (CHAPTER 3)

Samples of commercially produced traditional beer, as well as samples of indigenous homebrewed traditional beer, were collected from local brewers in selected settlements in the greater Kimberley area of the Northern Cape Province. The samples were consequently analysed for selected microbiota. In commercially produced and traditional homebrewed beer the mean counts for total coliforms and *Staphylococcus* spp. were *circa* 10^5 cfu.ml^{-1} whereas the respective TVC and total fungi counts were 10^6 and 10^7 cfu.ml^{-1}. In terms of total coliforms and *Staphylococcus* spp., homebrewed traditional beer counts were more-or-less one log-phase higher than the commercial product. The counts in the homebrewed beer likely originated from contamination during the handling process, whereas the commercial version was prone to contamination either in the raw ingredients or during post-processing and consumption. Apart from the occurrence of staphylococci, which indicated human handling, notable numbers of total coliforms indicated faecal contamination. To circumvent any hazards or health risks for the consumer, a basic knowledge of good manufacturing practices (GMP) and proper personal hygiene practices is necessary. An understanding of basic food safety and practices that prevent or reduce microbial growth is crucial for the reduction of food-borne illnesses. The implementation of legislative guidelines for traditional beverages, together with the monitoring and enforcing thereof by bodies such as the Department of Health and the Department of Environmental Health, should also receive priority.

6.4 SUMMATIVE REMARKS REGARDING THE PREVALENCE OF MYCOTOXINS (CHAPTERS 4 AND 5)

In chapters 4 and 5, the mycotoxins deoxynivalenol and citrinin were analysed in traditional beer samples using the Ridascreen deoxynivalenol (Fast) ELISA kit and Ridascreen citrinin (Fast) ELISA kit. Validation of the ELISA Ridascreen methodologies allowed for a more rapid, easier, reliable and accurate technique that could be used to quantify the level of mycotoxins (DON and citrinin) in the beer.

The deoxynivalenol level for the summer season was found to be above the recommended levels (2 ppb) set by the European Union, while citrinin levels in the samples varied between 35.6 ppb and 942.2 ppb. In the case of citrinin there were statistically significant differences between the spring, summer and winter samples. This observation highlights the seasonal impact on fungal growth and consequent mycotoxin production.

The presence of mycotoxins in the beer samples suggests a possible lack of storage and fermentation control, and the need to implement control and preventative measures is therefore underlined, especially in developing countries such as South Africa. The quality of the raw products used as ingredients is also questionable, particularly those supplied to poor communities and areas where proper control is not enforced.

6.4.1 Relationship between deoxynivalenol and citrinin

Figure 6.1 portrays the relationship between deoxynivalenol and citrinin in traditional beer. This was achieved by measuring the correlation (R^2) between the two mycotoxins. This evaluation was deemed important, because significant relationships might be indicative of:

- The co-occurrence of the mentioned compounds under similar conditions that will have an augmented effect on consumers;
- Synergism amongst certain fungal groups that predominate under particular conditions and in certain environments such as traditional beer and its ingredients; and
- The possibility of using specific mycotoxins as indicators of the general mycotoxin load – similar, for example, to coliforms that are applied as indicators of faecal contamination.

An R^2-value of 0.409 was noted between deoxynivalenol and citrinin (Figure 6.1), indicating a weak positive association. It can thus be deduced that a strong relationship between the mycotoxins does not exist under the specified conditions, and therefore the coexistence thereof in the product cannot necessarily be deduced. Analyses of the prevalence of these compounds would thus have to include methodologies that cover a wide spectrum of mycotoxins. This information also shows that environmental control aimed at minimising the occurrence of specific mycotoxins will not necessarily be effective at kerbing others.

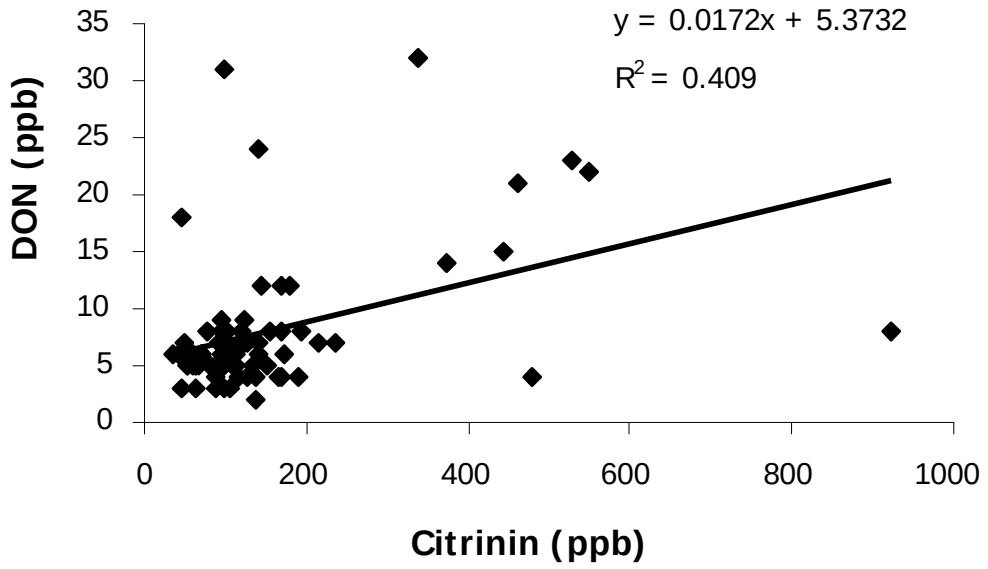

Figure 6.1: Relationship between deoxynivalenol and citrinin

(The statistical correlation (R^2) shows a weak positive association)

6.5 RECOMMENDATIONS

The fact that traditional beer is often consumed by immuno-compromised individuals emphasises the need to ensure a product free from microbial contamination and other harmful metabolites. Precautionary measures therefore need to be taken to ensure safety during the production, preparation, processing, storage and transportation of the product (WHO, 1996). Failure to adhere to such precautionary measures may have detrimental effects with regard to public health, which in turn will lead to poor publicity regarding the regions concerned. Intervention by local governments should be aimed at ensuring that minimum safety standards are upheld in terms of traditional beer preparation and consumption. This can be achieved by, inter alia, drafting regulations and specifications relating to traditional

beer, appropriating the necessary facilities for washing and cleaning (e.g. basins with hot and cold running water), protective clothing (hairnets and aprons), and commitment by brewers towards ensuring a safe and nutritious product.

In summary, the following measures should be considered:

- The establishment of educational programmes regarding hygienic personal and processing practices for brewers and consumers (refer to section 6.5.1);

- The registration of brewers and the issuing of licences;

- Proper monitoring and control of brewers by regulatory bodies;

- The implementation of basic HACCP principles to assist in the identification and control of potential hazards;

- The setting of legislative guidelines for mycotoxins in traditional beer; and

- The implementation and monitoring of good agricultural practices (GAP) that limit mycotoxins in raw products.

6.5.1 Proposed intervention in the form of an awareness programme to promote safety and hygiene during the production of traditional beer

As the study progressed it became evident that novel interventions should be sought and implemented in the area to address hazardous practices related to traditional beer. The following section proposes an awareness programme based on visual images coupled with supporting subscripts/slogans to carry across the fundamental aspects related to safe production and consumption. The programme is intended to be distributed in poster format in at least A3 size. These are to be distributed in prominent, visible locations in the townships and marginal urban areas bordering the city of Kimberley. The categories addressed in the poster include issues such as: 1) Personal and kitchen hygiene; 2) Safety and trustworthiness of ingredients for manufacturing; 3) Storage of ingredients; 4) Use of pure and safe water; 5) Cleanliness of utensils and containers; 6) The danger of adding hazardous substances to the beer; 7) Fresh consumption and proper storage of the beer; and 8) Guarding against offering beer to minors and preventing misuse.

The proposed hygiene awareness poster with accompanying subscripts is shown in Figure 6.2. The material developed utilises colour images, in animated format, of selected practices that need to be addressed. The images are accompanied by slogans that unambiguously summarise the particular image in the three dominant regional languages: English, Afrikaans and

Setswana. The considerations taken into account during compilation of the particular methodology were:

- The images had to be simple and clearly legible and directly address the most relevant hazards;
- The images had to be unambiguous, colourful, and non-discriminatory in terms of race and gender;
- The images had to be identifiable by residents in terms of lifestyle and socio-economic disposition; and
- The images had to follow a logical sequence in terms of the brewing and consumption steps (these are supported by chronological markings).

The images are augmented, in terms of acceptability/non-acceptability, by ✓ and ✘ markings.

Figure 6.2: Proposed poster to be used in a campaign aimed at promoting safety and hygiene in the production and consumption of traditional beer

Images are supported by written subscripts that briefly and unambiguously summarise the intent of the picture. By including written sections, the retention of the message in especially literate residents should be enhanced.

6.6 FUTURE RESEARCH

The study has opened up the following further research opportunities:

- Implementing the awareness programme proposed under section 6.5.1 and testing the effectiveness thereof;
- Conducting epidemiological surveys to relate specific diseases such as gastroenteritis, oesophageal cancer and kidney disease to microbial contamination and mycotoxin ingestion among consumers of traditional beer;
- Investigating the relationship between micro-organisms and mycotoxins in raw products used for traditional beer brewing;
- Expanding the study to other areas in Southern Africa;
- Testing the effectiveness of GAP methods to reduce mycotoxins in raw products.

6.7 REFERENCES

WHO (World Health Organisation). 1996. Essential safety requirements for street-vended foods. Available online: http://www.who.int/foodsafety/publications/fs_management/en/streetvend.pdf [Accessed 4 August 2003].