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Seasonal variation of citrinin in traditionally brewed African beer

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A seasonal study on citrinin concentrations in traditional beer was conducted using a standardised enzyme-linked immunosorbent assay. Representative samples of indigenous South African beer were collected during the hot, mild and cold seasons from marginal-urban settlements in the Kimberley area, South Africa. The mean temperature and humidity readings were 37°C and 29% for respectively summer, 19.4°C and 7.1% for spring, and 16.3°C and 6.5% for winter. Samples were analysed for the mycotoxincitrinin using a commercial Ridascreen (citrinin) ELISA kit, validated for the purpose 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 similar to the standard curves prepared using the kit standards. The mean citrinin concentrations found in the various samples were 257.6 µg/kg, 99.0 and 185.7 µg/kg for the summer, spring and winter seasons respectively and the levels ranged between 35.6 µg/kg (min) and 924.2 µg/kg (max). Statistically significant differences (p ≤0.05) were found between the citrinin levels of the summer and spring samples and also between citrinin levels of the spring and winter samples (p ≤0.001). The relatively high levels of citrinin in selected samples may, with prolonged exposure, have adverse impacts on the health of consumers. Thus further studies into the origin and levels of this mycotoxin should be a priority.

Key words: Citrinin, traditional beer, seasonal variation.

INTRODUCTION

Citrinin is a fungal metabolite and a common food contaminant (Flajs and Peraica, 2009) which can cause the deterioration of liver or kidney function in animals (Chan and Shiao, 2007; Sweeney and Dobson, 1998). Citrinin has been reported in foods by several authors and its presence, particularly in fermentation products, is thought to be a potential threat to public health (Bennett and Klich, 2003; Pitt et al., 1996). Krejci et al. (1996) found that food contaminated with *Penicillium citrinum* caused functional and morphological renal damage and resulted 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 widely recognised for producing this mycotoxin are *Penicillium citrinin*and *Penicillium expansion,* although *Penicillium verrucosum* is also known to produce citrinin (Watanabe, 2008). Fungal species that produce citrinin are predominantly mesophilic and grow at a temperature range of 5 to 40°C with an optimum of between 26 and 30°C.

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The production of citrinin occurs from 15 to 37°C (Roberts et al., 1996). Environmental factors such as temperature, humidity and rainfall also play a role in fungal growth which may result in mycotoxin production. Citrinin levels varying from 0.28 and 6.29 μg/g in *Monascus* fermentation 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 to 20000 $\mu g.kg^{-1}$ of Citrinin (Lillehoj and Goransson, 1980). Whilst several studies have also reported on the occurrence of mycotoxins in traditional beer produced from malt, as well as several other cereal-based commodities (Zinedine and Manes, 2009; Samar et al., 2007; Wolf-Hall, 2007; Odhav and Naicker, 2002; Scott, 1996). The maize used in the brewing process has frequently been reported to be contaminated with fungi (Hazel and Patel, 2004; Bullerman and Bianchini, 2007). Apart from maize, mycotoxin-producing fungi have been reported to also infect barley, wheat, corn, fruit and sorghum (Zinedine and Manes, 2009; Larsen et al., 2004). Most mycotoxins are chemically stable and as a result tend to survive storage and processing even at high temperature.

The aim of this study was to validate the Ridascreencitrinin enzyme-linked immunosorbent assay (ELISA) 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.

MATERIALS AND METHODS

Sampling Protocol

Seventy beer samples were collected from various traditional beer brewers during spring, summer, and winter in the greater Kimberley area, 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, mixed thoroughly 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.

Quantification of citrinin in beer samples using enzyme-linked immunosorbent assay (ELISA)

Ridascreencitrinin ELISA kits (AECI Amersham, South Africa) were used to analyse the mycotoxins and the manufacture`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, agitated and incubated for 10 min at room temperature. After incubation the plates were washed four times with 250 μl per well deionized H_20. The plates were then 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 unreacted antibodies were removed by washing three times with 250 μl of deionized H_20 per well. Two drops of chromogen substrate solution were added to each well, mixed and incubated in the dark for 5 minutes at room temperature after which the reaction was terminated by the addition of a stopping solution. The plate was read on a plate spectrophotometer at 450nm and the percentage absorbance was calculated for all the wells using the 0 ppb citrinin standard as the reference. The kit standards were used for the construction of a standard curve obtained by plotting percentage absorbance against citrinin concentrations. The resulting values were read from this standard curve.

Validation of the Ridascreen enzyme-linked immunosorbent assay (ELISA) for citrinin

The inter- and intra-assay variation of the Ridascreen ELISA for citrinin was determined to ascertain whether assays were reproducible. Samples were assayed in triplicate. The mean and standard deviations for the samples were calculated and the results were used to determine inter- and intra-assay variations.

RESULTS

Validation of the Ridascreen enzyme-linked immunosorbent assay (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 (data not shown). Figure 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.

The screening of traditional beer for citrinin using Ridascreen enzyme-linked immunosorbent assay (ELISA) kits

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, 257.6 and 185.7 μg/kg for the spring, summer, and winter seasons respectively and the levels ranged between 35.6 μg/kg (min) and 924.2 μg/kg (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 2).

DISCUSSION

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

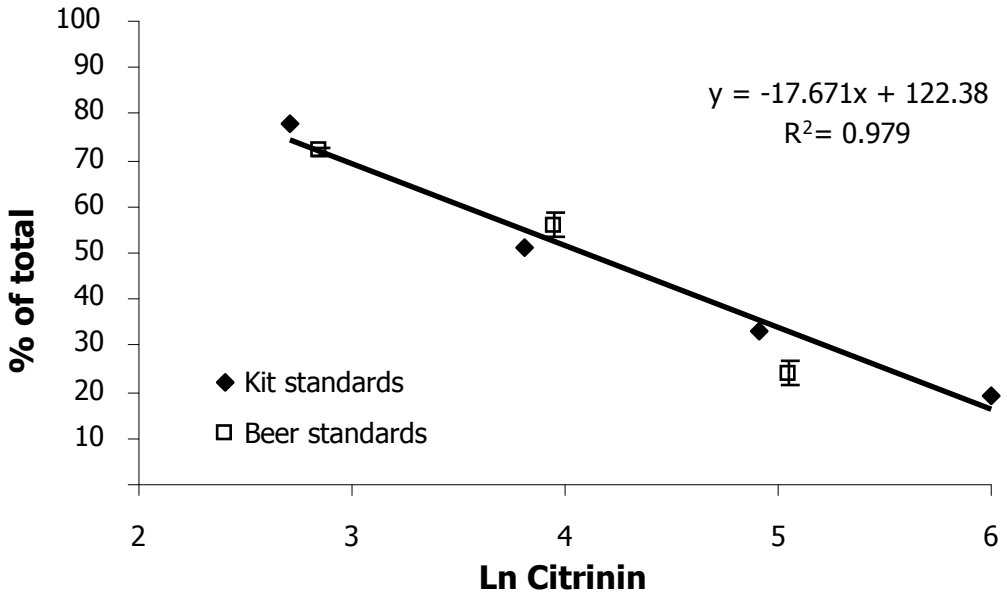

Figure 1. A comparison between the citrinin kit standard curve and standard curve using dilution series of indigenous traditional beer (Ln transformation).

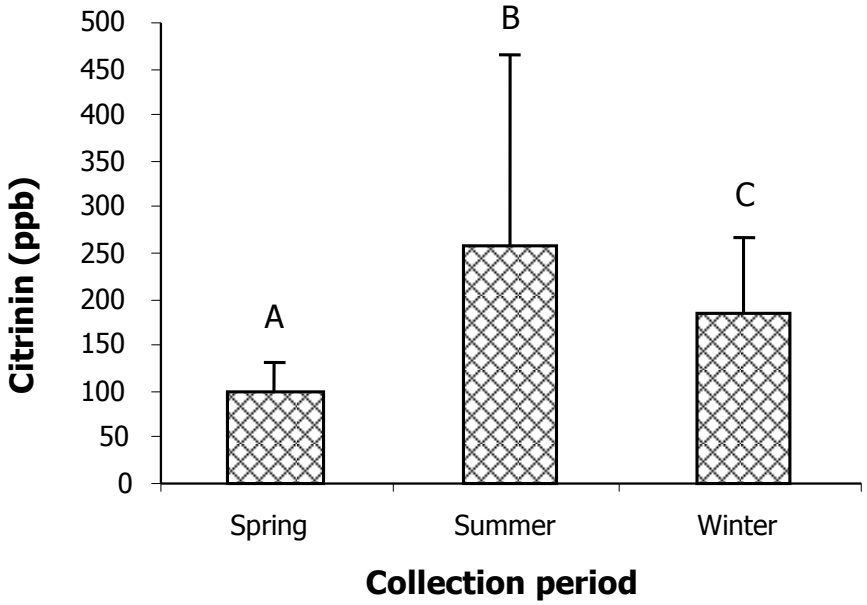

Figure 2. A comparison of the citrinin concentrations (mean) in samples collected at different seasons of the year (A/B significant $P \leq 0.05$; A/C significant $P \leq 0.001$).

there would be differences between the citrinin levels of the samples collected at these intervals. The citrinin levels in the samples collected during summer and spring, when the humidity and temperature were 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 (Schrodter, 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 (Magan et al., 2011; Raghavender and Reddy, 2009). The levels of citrinin detected in this study could pose serious health risks to consumers, especially considering citrinin can accumulate in the liver (Gao et al., 2010). Habitual consumers of these brewers therefore have greater exposure to the harmful effects of this myco-

toxin. Prevention of and/or monitoring of the production process in areas where traditional beer is brewed and consumed in large quantities are thus important.

REFERENCES

Bennett JW, Klich M (2003). Mycotoxins. Clin. Microbiol. Rev. 16: 497-516

Bullerman LB, Bianchini A (2007). Stability of mycotoxins during food processing. Intl. J. Food Microbiol. 119: 140-146.

Chan WH, Shiao NH (2007). Effect of citrinin on mouse embryonic development *in vitro* and *in vivo*. Reprod. Toxicol. 24: 120-125.

Flajs D, Peraica M (2009).Toxicological Properties of Citrinin. ArhivZaHigijenuRada I. Toksikologiju 60: 457-464.

Gao G, Karlgren M, Artursson P (2010). Uptake mechanism of ochratoxin A and citrinin through human liver. Drug Metabol. Rev. 42: 315.

Hazel CM, Patel S (2004). Influence of processing on trichothecene levels. Toxicol. Lett. 153: 51-59.

Krejci ME, Bretz NS, Koechel DA (1996). Citrinin produces acute adverse changes in renal function and ultrastructure in pentobarbital-anesthetized dogs without concomitant reductions in [potassium] (plasma). Toxicology 106: 167-177.

Larsen JC, Hunt J, Perrin I, Ruckenbauer P (2004). Workshop on trichothecenes with a focus on DON: summary report. Toxicol. Lett. 153: 1-22.

Lillehoj EB, Goransson B (1980). Occurrence of ochratoxin- and citrinin-producing fungi on developing Danish barley grain. Acta. Patho. I. Microbiol. Scand B 88: 133-137.

Liu BH, Wu TS, Su MC, Chung CP, Yu FY (2005). Evaluation of citrinin occurrence and cytotoxicity in Monascus fermentation products. J. Agric. Food Chem. 53: 170-175.

Magan N, Medina A, Aldred D (2011). Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathol. 60: 150-163.

Odhav B, Naicker V (2002). Mycotoxins in South African traditionally brewed beers. Food Addit.Contam. 19: 55-61.

Pitt JI, Teufel P, Tompkin RB (1996). The International Commission on Microbiological Specifications for Foods (ICMSF): Update. Food Control, 7: 99-101.

Raghavender CR, Reddy BN (2009). Human and animal disease outbreaks in India due to mycotoxins other than aflatoxins. World Mycotoxin. J. 2: 23-30.

Roberts TA, vanSchothorst M, Sharpe AN, BairdParker AC, Bryan FL, Buchanan RL, Busta FF, Doyle MP, Farkas J, Grau FH, Hobbs BC, Jouve JL, Mendoza S, Merican Z, Quevedo F, Pitt JI, Teufel P, Tompkin RB (1996). The International Commission on Microbiological Specifications for Foods (ICMSF): Update. Food Control 7: 99-101.

Samar M, Resnik SL, Gonzalez HHL, Pacin AM, Castillo MD (2007). Deoxynivalenol reduction during the frying process of turnover pie covers. Food Control 18: 1295-1299.

Schrodter R (2004). Influence of harvest and storage conditions on trichothecenes levels in various cereals. Toxicol. Lett. 153: 47-49.

Scott PM (1996). Mycotoxins transmitted into beer from contaminated grains during brewing. J. AOAC Int. 79: 875-882.

Sweeney MJ, Dobson ADW (1998). Mycotoxin production by Aspergillus, Fusarium and Penicillium species. Int. J. Fd. Microbiol. 43: 141-158.

Watanabe M (2008). Production of mycotoxins by Penicillium expansum inoculated into apples. J. Food Protect. 71: 1714-1719.

Wolf-Hall CE (2007). Mold and mycotoxin problems encountered during malting and brewing. Int. J. Food. Microbiol. 119: 89-94.

Zinedine A, Manes J (2009). Occurrence and legislation of mycotoxins in food and feed from Morocco. Food Control 20: 334-344.