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Fumonisin Mycotoxins in Traditional Xhosa Maize Beer in South Africa

GORDON S. SHEPHARD,* LIANA VAN DER WESTHUIZEN, PUMZA M. GATYENI, NONTUTHUZELO I. M. SOMDYALA, HESTER-MARI BURGER, AND WALTER F. O. MARASAS

PROMEC Unit, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa

The production and consumption of home-brewed Xhosa maize beer is a widespread traditional practice in the former Transkei region of South Africa. HPLC determination of fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) in maize beer samples collected in two magisterial areas, Centane and Bizana, showed a wide range of levels. All samples were positive for FB_1, with a mean level of 281 ± 262 ng/mL and a range from 38 to 1066 ng/mL. Total fumonisins ($FB_1 + FB_2 + FB_3$) ranged from 43 to 1329 ng/mL, with a mean of 369 ± 345 ng/mL. Data on the consumption of home-brewed beer are not available. On the basis of published data for the consumption of commercial beer in South Africa, the fumonisin exposure in these districts among the consumers of maize beer was found to be well above the provisional maximum tolerable daily intake of 2 μg/kg of body weight/day set by the Joint FAO/WHO Expert Committee on Food Additives.

KEYWORDS: Fumonisins; mycotoxins; Transkei; beer; maize; corn; *Fusarium*

INTRODUCTION

The fumonisin mycotoxins, mainly produced by the fungi *Fusarium verticillioides* (Sacc.) Nirenberg and *Fusarium proliferatum* (Matsushima) Nirenberg, occur widely in maize and maize-based products (*1*). Although at least 28 different analogues are known, the most abundant naturally occurring forms are fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) (*2*). They are the causative factors of certain animal disease syndromes such as leukoencephalomalacia in horses and pulmonary edema in swine (*3*, *4*). The fumonisins are hepato- and nephrocarcinogenic in rats and mice (*5*, *6*). The International Agency for Research on Cancer (IARC) has classified FB_1 as a group 2B carcinogen (possibly carcinogenic to humans) (*7*). Both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food of the European Commission have assigned a provisional maximum tolerable daily intake (PMTDI) for FB_1, FB_2, and FB_3, either alone or in combination, of 2 μg/kg of body weight (bw)/day (*8*, *9*). The fumonisins have been statistically associated with the high incidence of esophageal cancer in the Centane magisterial area of the former Transkei region of South Africa (*10*) and in Linxian County, Henan Province, and Cixian County, Hebei Province, China (*11*, *12*). The exposure of these rural subsistence farming communities is a concern due to the high contamination levels of maize in these regions, as well as the excessive reliance of these communities on maize as a dietary staple (*13*).

The presence of fumonisins in commercial clear beers was first reported by Scott and Lawrence (*14*), who found that of 41 local and imported beers sampled in Canada, 4 showed levels >2 ng of FB_1/mL (levels of 7.8, 15, 22, and 59 ng of FB_1/mL), whereas a further 7 contained detectable levels of ∼1 ng of FB_1/mL. The origin of the fumonisins in these beers was presumed to be fumonisin-contaminated maize or maize product used as a brewing adjunct. Previous studies on the fate of fumonisins during fermentation of maize for the production of ethanol had shown that little degradation of fumonisin occurred during the 3-day fermentation process and that most of the initial fumonisin was recovered in distillers' grains, thin stillage, and distillers' solubles fractions (*15*). Studies in which FB_1 and FB_2 were added to wort and fermented using three different strains of *Saccharomyces cerevisiae* for up to 8 days indicated small losses of FB_1 and FB_2 of 3−28% and 9−17%, respectively (*16*). The use of maize spiked with fumonisin-containing *F. verticillioides* culture material to make joala, a local traditional maize beer in Lesotho, demonstrated that fumonisins are extracted into the beer and that the beermaking process did not affect toxicity as shown by short-term rodent studies (*17*). Further surveys of local and imported commercial beers in Canada, Spain, Kenya, and the United States have been reported (*18*−*21*). Using a sensitive indirect competitive ELISA for fumonisins, Scott et al. screened 46 beers from the local Canadian market and found 22 to be contaminated at levels between 0.2 and 24.7 ng/mL, with 21 of these contaminated at levels of <4.8 ng/mL (*18*). Competitive ELISAs were used in two other surveys of beer. In Spain, 32 beer samples, including nonalcoholic beers, showed contamination in 14 samples at levels between 4.76 and 85.53 ng/mL (*19*), whereas in Kenya, 75 samples from two brands of commercial clear lager beers showed a contamination rate of

* Author to whom correspondence should be addressed (telephone +27-21-938-0279; fax +27-21-938-0260; e-mail gordon.shephard@mrc.ac.za).

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Table 1. Fumonisin (FB) Levels in Home-Brewed Maize Beer Collected in Various Locations within the Districts of Bizana and Centane of the Former Transkei Region of South Africa

		ng/mL			
year	source	FB_1	FB_2	FB_3	total FB
2003	Nomlacu, Bizana	38	20	nd[a]	58
2003	Nomlacu, Bizana	302	49	10	361
2003	Nomlacu, Bizana	313	68	8	389
2003	Nomlacu, Bizana	40	12	nd	52
2003	Madadana, Bizana	259	73	14	346
2003	Madadana, Bizana	62	8	nd	70
2003	Madadana, Bizana	43	nd	nd	43
2003	Madadana, Bizana	43	9	nd	52
2003	Madadana, Bizana	255	83	16	354
2003	Madadana, Bizana	61	14	nd	75
2001	Umnyaka, Bizana	1066	135	128	1329
2004	Qolora, Centane	273	39	24	336
2003	Mtwaku, Centane	153	40	11	204
2001	Mtwaku, Centane	515	77	38	630
1992	Qolora, Centane[b]	610	255	75	940
1991	Maxhama, Centane[b]	341	140	46	527
1991	Feni, Centane[b]	473	102	43	618
1991	Chwebeni, Centane[b]	210	41	10	261
mean ± SD of positive samples		281 ± 262	69 ± 64	35 ± 36	369 ± 345

[a] Not detected (<5 ng/mL). [b] Reference *24*.

72% at levels of up to 0.78 ng/mL (*20*). A survey using a HPLC method for fumonisin determination was conducted on 29 beers purchased in Lincoln, NE, and showed 25 (86%) of the samples to be positive for FB_1 and 41% to be positive for FB_2 (*21*). The total fumonisin ($FB_1 + FB_2$) content ranged from 0.3 to 12.7 ng/mL, and the mean of the positive samples was 4.0 ng/mL.

The home brewing of maize-based beer is widely practiced and enjoyed in rural subsistence farming areas of South Africa. Preparation of the beer is mostly performed by the rural women who utilize home-grown maize as the initial ingredient. Distinct from the commercial process of production of clear beers using barley malt and hops, this production of alcoholic liquor follows traditional practices and results in an opaque beverage containing a maize-based suspension of solids. The traditional process involves an initial fermentation, cooking, and a second fermentation for between 1 and 2 days, before the mixture is coarsely sieved for the removal of large solids. Moldy home-grown maize, specifically separated from good (visibly nonmoldy) maize by the householders after harvest, is frequently used as a component, and it is said to impart a desirable taste to the final beer product.

The aim of this study was to determine the fumonisin levels in samples of home-brewed maize-based beers prepared by the Xhosa-speaking population in the former Transkei region of the Eastern Cape Province of South Africa and to estimate the extent to which this could contribute to fumonisin exposure of this population.

MATERIALS AND METHODS

Samples. Samples of home-brewed maize-based beer (known as mqomboti in the local isiXhosa language) were obtained prior to various local "drinking parties" in the Centane and Bizana magisterial districts of the former Transkei region of the Eastern Cape Province of South Africa during the period from 2001 to 2004 and stored at −20 °C prior to analysis. **Table 1** gives details of collection place and time for all samples.

Reagents and Standard. Fumonisin standards were prepared in the PROMEC Unit, Tygerberg, South Africa, according to the method of Cawood et al. (*22*). All solvents and chemicals were of analytical grade from Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Millipore, Bedford, MA).

Determination of Fumonisins. The analytical method for the determination of fumonisins in maize-based beer was adapted from the method for maize (*23*). In brief, beer (75 mL) was mixed with methanol (25 mL) and homogenized for 3 min. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant filtered (Macherey-Nagel MN 617). An aliquot (10 mL) was adjusted to pH 3 by the addition of 1 M hydrochloric acid and then loaded on a reversed-phase (C_{18}) solid phase extraction cartridge previously conditioned with methanol (5 mL) and water (5 mL). The column was washed with water (3 mL) and methanol/water (1:3, 5 mL) prior to elution of fumonisins with methanol (15 mL). The eluate was evaporated to dryness under a stream of nitrogen at 60 °C and the dried residue stored at 4 °C. Sample residues were redissolved in methanol and aliquots derivatized with *o*-phthaldialdehyde (OPA) and determined by reversed-phase HPLC using a Waters (Milford, MA) Breeze system coupled to a Waters 474 fluorescence detector set at excitation and emission wavelengths of 335 and 440 nm, respectively. The fumonisins were separated on a Phenomenex (Torrance, CA) Synergi 4 μm MAX-RP column (75 × 4.6 mm) using a mobile phase of methanol/0.1 M sodium phosphate buffer (73:27, pH 3.35) pumped at a flow rate of 1 mL/min. Quantification was by peak area comparison with a similarly derivatized standard.

The method was validated in-house with respect to precision and recovery. Five replicate analyses of a single beer sample containing mean FB_1, FB_2, and FB_3 levels of 364, 99, and 14 ng/mL gave relative standard deviations of 4.7, 5.5, and 2.7%, respectively. Triplicate recoveries of FB_1, FB_2, and FB_3 from a beer sample spiked at a level of 1000 ng/mL for each analogue showed mean recoveries of 91, 91, and 99%, respectively.

Differences between samples collected in Centane and Bizana were tested for statistical differences by a comparison of means using an independent-samples *t* test (equal variances not assumed) at $P = 0.05$ (SPSS version 12, Chicago, IL).

RESULTS AND DISCUSSION

The levels of FB_1, FB_2, FB_3, and total fumonisins in Transkeian traditional home-brewed maize beer are shown in **Table 1**. Included in this set of results are previous HPLC determinations of fumonisins in beer samples collected in various locations in Centane (*24*). The total fumonisin ($FB_1 + FB_2 + FB_3$) levels ranged from 43 to 1329 ng/mL with a mean of 369 ± 345 ng/mL, and as a consequence the standard deviation of the mean is large. As found in surveys of maize (*1*, *8*), FB_1 was the major fumonisin analogue present and accounted for a mean of 76% (range of 65−84% of the total) in samples containing all three analogues. When the data are separated into beer samples from Centane and Bizana, mean total fumonisin levels were 284 ng/mL in Bizana and 502 ng/mL in Centane. This difference mirrors the situation with respect to fumonisins in home-grown maize, in which it has been shown that levels in Centane are generally higher than levels in Bizana (*10*). However, the range of levels in beer observed in both districts was large, and statistical analysis revealed that these differences were not significant ($p > 0.05$). The fumonisin levels in these samples were roughly 2 orders of magnitude greater than levels observed in the surveys of clear commercial beers purchased in Canada and the United States in which maize or maize products are used merely as adjuncts (*14*, *18*, *21*). Apart from the published data on commercial clear lager beer in Kenya, which also showed the presence of deoxynivalenol and zearalenone (*20*), very little information is available on mycotoxin contamination of beer in Africa. Sporadic reports have shown the presence of aflatoxins and zearalenone in beers prepared from maize, millet, or sorghum in Kenya, Lesotho, Nigeria, South Africa, and Zambia (*25*).

The large range of fumonisin levels observed in these beer samples reflects differences in the quality of maize used in their

preparation, especially in the possible use of home-grown moldy maize as an ingredient. It is a universal custom in these areas to hand sort each maize harvest into good (visibly nonmoldy) and moldy maize cobs, a practice that is expected to reduce fumonisin exposure. However, the use of the moldy maize for beer brewing obviates the food safety benefit derived from the original separation. During the period from 2001 to 2004 when the samples were being collected, a survey was conducted in the Centane and Bizana magisterial areas to ascertain the extent to which moldy maize is used for various purposes in households in these Transkeian districts. In total, 88 households in Bizana and 48 households in Centane provided data. In both areas, all households surveyed separated their home-grown maize harvest into good and moldy fractions. In Bizana, 50% of households (44/88) reportedly used moldy maize in beer brewing, whereas in Centane, only 25% (12/48) admitted to this practice. It has been widely assumed that the moldy maize may be consumed as food by householders once good maize is finished. However, in each area, only one household of those surveyed acknowledged this practice, and the majority (43/48 and 85/88 households in Centane and Bizana, respectively) bought commercial maize once the good maize of the home-grown crop was exhausted. Apart from its widespread use in maize beer, householders reported that they either discard the moldy maize or give it to animals to eat.

To assess fumonisin exposure due to the drinking of maize beer in rural areas, it is necessary to know the level of beer consumption in Africa. This is a statistic not readily derived. A study of aflatoxin contamination of locally brewed traditional alcoholic beverages in Dar es Salaam, Tanzania, indicated that these beverages, which contain relatively low amounts of alcohol, are sometimes consumed in large quantities of up to 5−6 L/day by local inhabitants (*26*). In an international comparison of commercial beer consumption for 1999, the Brewers Association of Japan estimated per capita annual beer consumption in South Africa as 54.3 L/year, equivalent to 149 mL/day (*27*). This is similar to the estimated per capita consumption of 58 L/year (159 mL/day) for commercial beer given in 2005 by the predominant commercial brewer in South Africa (*28*). More useful than per capita statistics, the Report on South African Food Consumption Studies Undertaken amongst Different Population Groups (1983−2000) provides additional statistics for commercial beer consumption (*29*). The report combines results from various South African studies and summarizes the portions consumed per day for individual food items both on a per capita basis and by individuals who actually consume the relevant item. Although the per capita commercial beer consumption reported is only 32 mL/day, lower than that reported by commercial brewers, the report gives beer consumption among beer consumers ("drinkers") as 1048 mL/day with a 97.5th percentile of 4125 mL/day. Estimates of fumonisin exposure due to maize beer drinking based on these consumption figures for commercial beer in South Africa are shown in **Table 2**. The PMTDI of 2 μg/kg of bw/day derived by JECFA provides a guide for risk characterization (*8*). Clearly in the case of the per capita consumption, exposures, although a significant fraction of PMTDI, are less than the 2 μg/kg of bw/day limit, but would rise above it for consumption of the more contaminated beers in this survey. More realistically, the beer consumption of "drinkers" needs to be examined. Fumonisin exposure of these resulting from the consumption of beer at the mean total fumonisin level found in this survey would be >3 times the PMTDI. Exposures for the 97.5th percentile of the population of "drinkers" would be even higher, especially if the more contaminated beers found in the survey were to be consumed. Combined with possible fumonisin exposure from the maize-based staple diet, these estimates are a cause for concern.

Table 2. Fumonisin (FB) Exposure Due to Maize Beer in Various Locations within the Districts of Bizana and Centane in the Former Transkei Region of South Africa Using Various Estimates of Beer Consumption

beer consumption	mL/day	total FB (ng/mL)	fumonisin exposure	
			μg/person/day	μg/kg of bw/day[c]
per capita (*27*)	149	369[a]	55	0.9
per capita (*28*)	159	369[a]	59	1.0
per capita (*29*)	32	369[a]	12	0.2
consumers only				
mean (*29*)	1048	369[a]	387	6.5
97.5th% (*29*)	4125	369[a]	1522	25.4
97.5th% (*29*)	4125	1329[b]	5482	91.4

[a] Mean of samples surveyed. [b] Maximum of samples surveyed. [c] Calculated by assuming a 60 kg individual. The Report on South African Food Consumption Studies Undertaken amongst Different Population Groups (1983–2000) gives mean body weight of adults (>10 years) as 55.7 or 60.9 kg, depending on the meta-analysis method used (*29*).

The results of this research indicated that the consumption of maize beer in rural areas of the former Transkei, South Africa, can significantly enhance fumonisin exposure and that, among beer consumers, the PMTDI set by JECFA can be readily exceeded. The situation is exacerbated by the high prevalence of the use of moldy maize as an ingredient of choice and can be addressed only by education and lifestyle change.

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