# Occurrence of Deoxynivalenol and Nivalenol in *Fusarium graminearum* Infected Undergrade Wheat in South Africa

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Symptoms of head blight (scab) of wheat were observed on four farms in the southern Cape region of South Africa in November 1987. *Fusarium graminearum* group 2 was found to be the causative fungus of this disease, which has not been reported in South Africa until recently. Samples of undergrade wheat severely affected with scab were obtained from three farms and analyzed for the presence of deoxynivalenol and nivalenol by capillary gas chromatography and for zearalenone by high-performance liquid chromatography. The trichothecenes deoxynivalenol and nivalenol were present in all samples at levels of 3750–14 360 and 320–1850 ng/g, respectively. Zearalenone could not be detected in any of the samples. The presence of the two trichothecenes was also demonstrated by a liquid chromatographic procedure and verified by capillary gas chromatography using selected ion monitoring mass spectrometry.

*Fusarium graminearum* Schwabe, the conidial state of *Gibberella zeae* (Schw.) Petch, has been differentiated into two populations designated as groups 1 and 2 (Burgess et al., 1975; Francis and Burgess, 1977). Members of group 1 are usually associated with diseases of the crowns of plants and do not form perithecia in culture except in compatible crosses according to Francis and Burgess (1977). Members of group 2 are usually associated with diseases of aerial parts of plants and readily form perithecia in single-conidial cultures (Francis and Burgess, 1977). Thus, it has been widely accepted that crown rot of wheat is caused by *F. graminearum* group 1, whereas head blight is caused by *F. graminearum* group 2 (Burgess et al., 1981; Nelson et al., 1983). *F. graminearum* is known to produce a number of mycotoxins including the type B trichothecenes deoxynivalenol (DON) and nivalenol (NIV) as well as the estrogenic metabolite zearalenone (Figure 1A–C). Animals consuming zearalenone-contaminated feed develop reproductive problems (Marasas et al., 1984), while trichothecene intoxication of man and animals is characterized by skin lesions, vomiting, diarrhea, and damage to hematopoietic tissues (Ueno, 1983; Marasas et al., 1984).

The natural occurrence of zearalenone in various cereals has been widely reported (Marasas et al., 1984). The presence of DON as a major contaminant in head-blighted or "scabby" wheat has been reported in Canada (Trenholm et al., 1981, 1983; Scott, 1983; Scott et al., 1984), the United States (Eppley et al., 1984; Hagler et al., 1984; Seitz and Bechtel, 1985; Shotwell et al., 1985), Poland (Visconti et al., 1986), the United Kingdom (Osborne and Willis, 1984), and Sweden (Pettersson et al., 1986). DON also occurs as a major contaminant in other substrates, especially corn (Jemmali et al., 1978; Vesonder et al., 1979; Vesonder and Ciegler, 1979; Gilbert et al., 1983; Vesonder 1983). The cooccurrence of NIV and DON in wheat has been documented in Japan (Kamimura et al., 1981; Yoshizawa 1983; Tanaka et al., 1985b,c), Korea (Lee et al., 1985, 1986), the United Kingdom (Tanaka et al., 1986), Poland (Ueno et al., 1985), and Taiwan (Ueno et al., 1986). An assessment of the extent of NIV contamination in foods and feeds is essential, since its toxicity is higher than that of DON and is of the same order as that of the potent type A trichothecene T-2 toxin (Ueno, 1984).

Due to the potential health problems posed by the contamination of food and feedstuffs with these *Fusarium* mycotoxins, various analytical procedures utilizing gas and liquid chromatography have been developed for the determination of zearalenone (Chang and De Vries, 1984; Bagneris et al., 1986) and NIV and DON (Kamimura et al., 1981; Chang et al., 1984; Tanaka et al., 1985a; Trenholm et al., 1985; Scott et al., 1986; Lauren and Greenhalgh, 1987) in various food and feed substrates.

Head blight of wheat has not been recorded in South Africa until recently (Scott et al., 1988). During 1987, an outbreak of the disease caused by *F. graminearum* group 2 occurred in a small area of the southern Cape Province (Marasas et al., 1988). Samples of scabby, undergrade wheat were obtained from three farms. The chemical analyses of these three wheat samples for DON, NIV, and zearalenone are reported in this paper.

## EXPERIMENTAL SECTION

**Apparatus.** The gas chromatograph (GC) used was a Varian Model 3700, equipped with a $^{63}Ni$ electron capture detector, splitless injector, and a 30 m × 0.32 mm (i.d.) DB-5 fused silica capillary column. Liquid chromatographic (HPLC) separations were performed on a 4-μm Nova Pak $C_{18}$ column (15 cm × 3.9 mm (i.d.)) using a Waters Model 510 liquid chromatographic pump. Peak detection was performed on either a LDC ultraviolet (UV) detector for NIV/DON analyses or a Perkin-Elmer 650S fluorescence detector for zearalenone analyses. A Hewlett-Packard 5890A GC coupled with a mass-selective detector (MSD) and fitted with a 12 m × 0.2 mm (i.d.) HP-1 capillary column was used for verification purposes.

**Analytical Standards.** DON was isolated within the Institute, zearalenone was obtained from Makor Chemicals, and NIV was obtained from Wako Chemicals. Each standard was assessed for purity by either HPLC or GC. Only a single component was detected in each standard.

**Procedures.** *Wheat Samples.* Following harvesting and grading of the wheat crops in December 1987, a sample (70 kg) of undergrade wheat was obtained from each of the three farms in the southern Cape Province where head blight caused by *F. graminearum* group 2 occurred (Marasas et al., 1988). Subsamples (1 kg) were stored at 5 °C prior to chemical analyses.

*Determination of DON and NIV.* Samples were analyzed for the presence of DON and NIV according to the methods of both Scott et al. (1986) and Lauren and Greenhalgh (1987).

The method of Scott et al. (1986) may be summarized as follows. Subsamples (50 g) were extracted with methanol/water (7:3). Proteinaceous material was precipitated with ammonium sulfate and the solution filtered. An aliquot of the filtrate was partitioned into ethyl acetate (on a hydrophilic matrix), which was evaporated

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**Table I. Chromatographic Conditions for the Analysis of NIV, DON, and Zearalenone**

| parameter | LC | | GC-ECD: NIV/DON | GC-MSD: NIV/DON |
|---|---|---|---|---|
| | NIV/DON | zearalenone | | |
| mobile phase | MeOH/$H_2O$ (14:86) | MeOH/$H_2O$ (70:30) | helium | helium |
| flow rate | 0.8 mL/min | 0.5 mL/min | 35 cm/s | 39 cm/s |
| injection vol, μL | 20 | 20 | 1 | 1 |
| makeup gas | | | nitrogen | |
| makeup flow, mL/min | | | 48 | |
| injector temp, °C | | | 180 | 200 |
| detector temp, °C | | | 300 | 300 |
| oven profile (initial temp, °C) | | | 80 (1 min) | 50 (1 min) |
| (ramp (°C/min), final temp, °C) | | | 10–275 | 10–250 |
| detector wavelength, nm | 229 | 320 (ex)<br>445 (em) | | |
| attenuation | 0.004 AUFS | | $16 \times 10^{-12}$ A/mV | |
| detection limit, ng/g | 15 (NIV), 50 (DON) | 10 | 20 (NIV, DON) | |

**Table II. Comparison of the Percentage of Wheat Kernels Infected by *F. graminearum* Group 2, Seedling Survival and Levels of Nivalenol, Deoxynivalenol, and Zearalenone Determined by either Capillary Gas or Liquid Chromatographic Procedures**

| farm no. | kenels infected by *F. graminearum*[a], % | survival of seedlings,[a] % | GC[b] | | LC:[c] |
|---|---|---|---|---|---|
| | | | nivalenol, ng/g | deoxynivalenol, ng/g | zearalenone, ng/g |
| 1 | 40 | 46 | 320 | 3750 | ND |
| 2 | 65 | 9 | 1850 | 14360 | ND |
| 3 | 60 | 20 | 640 | 7980 | ND |

[a] Data from Marasas et al. (1988). [b] According to the method of Scott et al. (1986). [c] According to the method of Bagneris et al. (1986).

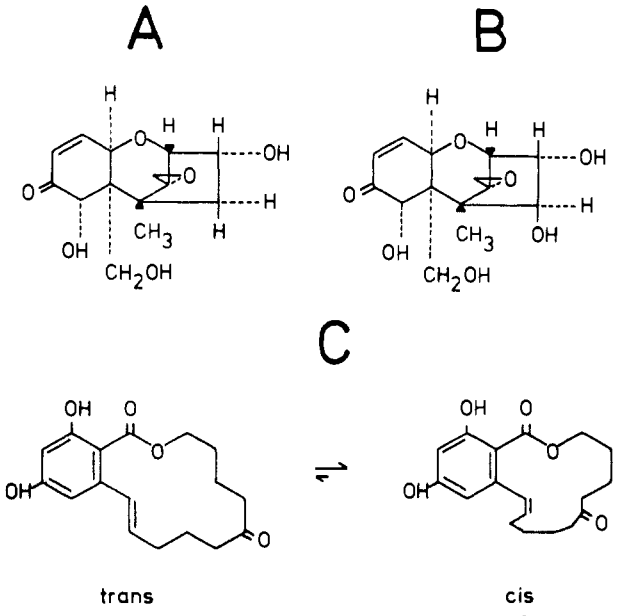


**Figure 1.** Chemical structures of (A) deoxynivalenol, (B) nivalenol, and (C) zearalenone.

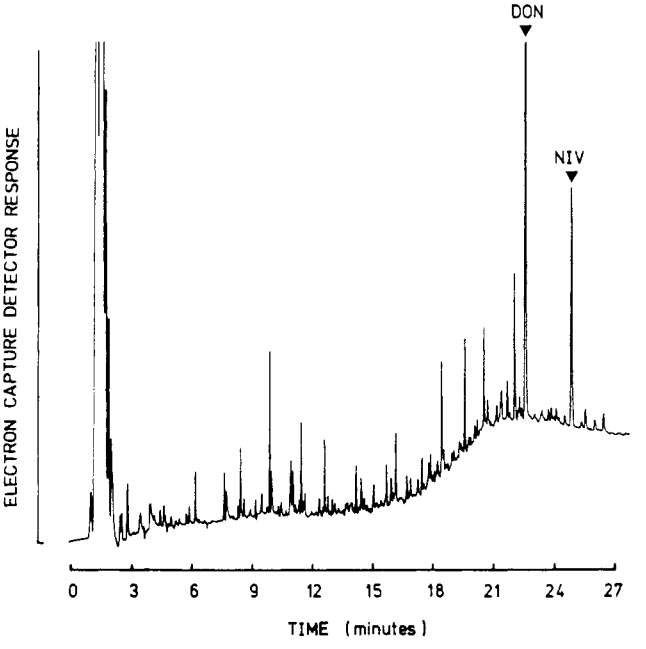


**Figure 2.** Gas chromatogram of a TMS-derivatized sample extract contaminated with DON (22.3 min) and NIV (24.6 min).

to dryness. The residue was further purified by chromatographic separation on a short silica gel column. The separately prepared trimethylsilyl (TMS) and heptafluorobutyryl (HFB) derivatives of the purified extracts were then analyzed by capillary GC.

The method of Lauren and Greenhalgh (1987) involved extraction of a 20-g subsample with acetonitrile/water (85:15) and primary purification on a cation-exchange/alumina–carbon column, followed by secondary purification on a carbon–Celite column. The resultant extracts were then analyzed by HPLC using UV detection.

*Determination of Zearalenone.* The wheat samples were screened for the presence of zearalenone according to the method of Bagneris et al. (1986), which involves extraction of a 30-g subsample with chloroform/water (25:2) followed by primary partitioning into a base solution and, after acidification, secondary partitioning into dichloromethane. The purified extracts were then analyzed by HPLC coupled with fluorescence detection for quantitative purposes.

*Chromatographic Procedures.* The exact chromatographic conditions used for the determination of each mycotoxin are described in Table I. Quantification by both GC and HPLC procedures was done by comparison of the peak height of each toxin against a calibration curve of the peak heights obtained with the respective authentic standards. Each determination was performed in duplicate, and each positive extract was spiked with standards and rechromatographed for confirmation purposes.

## RESULTS AND DISCUSSION

Since *F. graminearum* group 2 was the predominant fungus isolated from the wheat samples (Marasas et al., 1988), these samples were screened for the presence of the type B trichothecenes (NIV and DON) and zearalenone. Figure 2 shows the presence of the TMS derivatives of both DON and NIV in one of the sample extracts analyzed by the GC procedure of Scott et al. (1986), and the quantitative results for all three samples are presented in Table II. Gas chromatographic analyses of the HFB derivatives

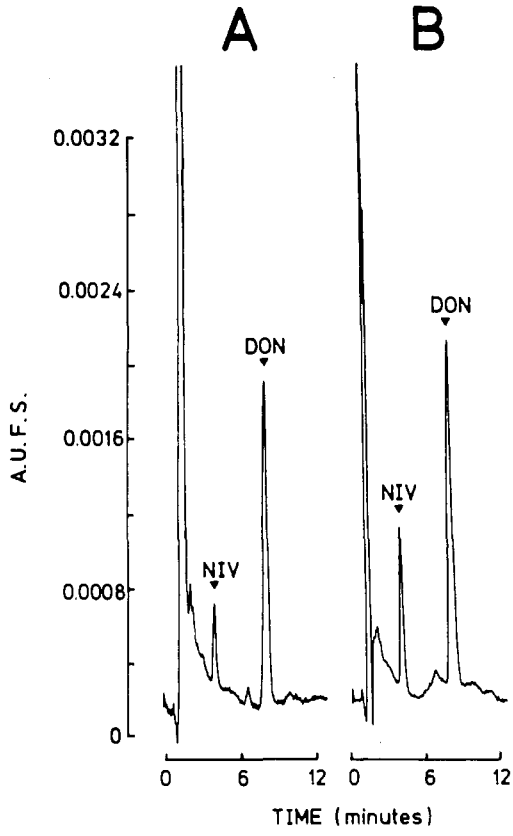


**Figure 3.** Liquid chromatogram of (A) a sample extract prepared according to the method of Lauren and Greenhalgh (1987) and (B) the same extract spiked with authentic NIV and DON standards.

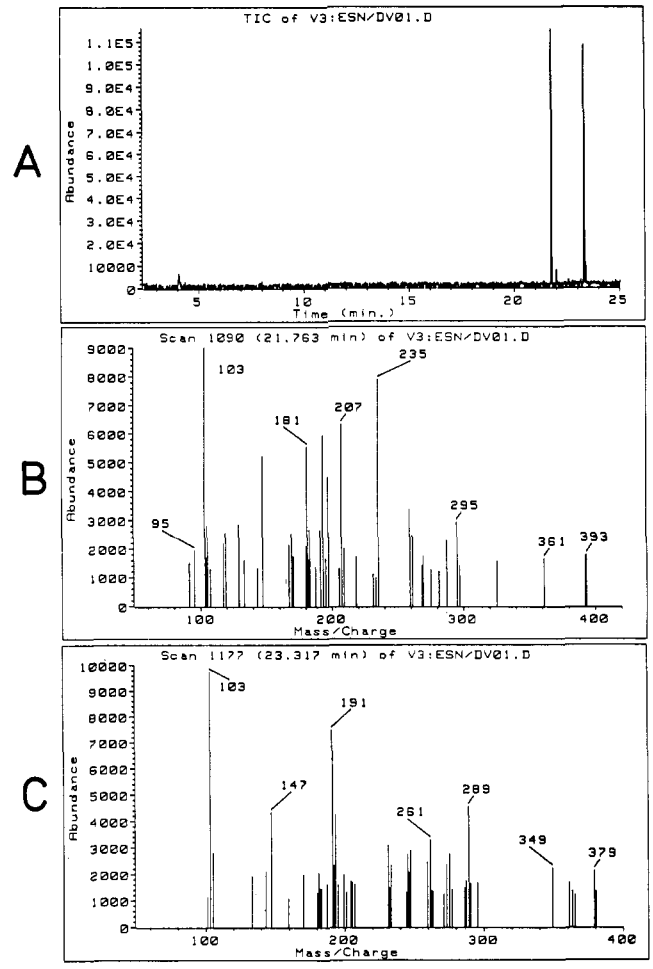


**Figure 4.** (A) Total ion chromatogram of 5 ng each of DON and NIV as their respective TMS derivatives, (B) mass spectrum of TMS-DON eluting at 21.76 min, and (C) mass spectrum of TMS-NIV eluting at 23.32 min.

of the sample extracts similarly showed the presence of both DON and NIV in each sample. Further evidence for the presence of these trichothecenes in all samples was obtained by the qualitative determination of DON and NIV in each sample using the HPLC method of Lauren and Greenhalgh (1987). Figure 3A shows the HPLC chromatogram obtained from one of the sample extracts, while the positive spike of the same extract with authentic DON and NIV standards is shown in Figure 3B. It should be stressed that a positive spike need not necessarily be accompanied by an increase in peak height; rather, the observation of a single peak in a spiked sample may be considered as confirmatory evidence for the compound's presence.

In order to unequivocally verify the presence of NIV and DON in the samples, an extract from one of the samples (prepared according to the method of Scott et al. (1986)) was subjected to capillary GC–mass spectrometry. Figure 4A shows the total ion chromatogram (mass range $m/z$ 80–500) of 5 ng each of DON and NIV in a calibration sample as their respective TMS derivatives. Figure 4B shows the "partial" mass spectrum (major ions only) of DON eluting at 21.76 min, and Figure 4C, a similar mass spectrum of NIV eluting at 23.32 min. From these data, ions specific to DON and NIV were chosen for the selected ion monitoring (SIM) analysis of the sample extract (necessary due to the relatively low concentrations of NIV and DON present in the sample extract). Figure 5A shows the total ion chromatogram of the sample extract monitored at two sets of three ions each. The relative intensities of the three masses monitored in the derivative peaks eluting at 21.77 and 23.32 min are shown in parts B and C, respectively, of Figure 5. The excellent agreement of retention times, coupled with the presence and relative intensities of all monitored ions (each set conspecific with those obtained from authentic standards), verified the presence of both DON and NIV in the sample extract.

No zearalenone could be detected in any of the samples by the method of Bagneris et al. (1986). Comparisons of the levels of DON and NIV determined in the three samples, the degree of infection of the kernels by *F. graminearum*, and the subsequent survival of seedlings after 14 days in a growth medium (Marasas et al., 1988) are given in Table II. The observed levels of trichothecene contamination in each sample are in accordance with the mycological results of Marasas et al. (1988) (Table II). The wheat sample from farm 2 not only had the highest levels of DON and NIV but also had the highest level of kernel infection by *F. graminearum* and the lowest seedling survival rate. The results from the other two farms were similarly correlated.

The cooccurrence and the range of levels of NIV, DON, and zearalenone detected in wheat grains and products reported from a number of countries are summarized in Table III. The levels of NIV determined in the present study (Table II) are comparable to those levels previously published. The levels of DON detected in the wheat samples analyzed in this investigation (3750–14 360 ng/g) are higher than any of the levels reported in Table III, where NIV and zearalenone were cocontaminants. However, it should be stressed that the wheat samples from the southern Cape Province of South Africa were undergrade, noncommercial samples from farms severely affected by *F. graminearum* head blight (Marasas et al., 1988). Visconti et al. (1986) reported even higher levels

**Table III. Nivalenol, Deoxynivalenol, and Zearalenone in Wheat Grains and Products: Levels of Contamination and Percentage of Samples Positive[a]**

| country | type | reference | NIV,[b] ng/g | DON,[c] ng/g | ZEA,[d] ng/g |
|---|---|---|---|---|---|
| Japan | grain | Tanaka et al. (1985a) | 47–439 (39) | 704–6920 (100) | 8–706 (100) |
| Japan | flour | Tanaka et al. (1985b) | 4–84 (33) | 2–239 (72) | 1–6 (11) |
| Korea | grain | Lee et al. (1985) | 28–184 (20) | 18–95 (100) | 8–40 (20) |
| Korea | grain | Lee et al. (1986) | 82–3169 (100) | 6–173 (56) | 3–1254 (56) |
| Poland | grain | Ueno et al. (1985) | 3–350 (90) | 7–309 (27) | 0–76 (2) |
| Taiwan | grain | Ueno et al. (1986) | 5–169 (45) | 26–2460 (59) | 4–32 (41) |
| U.K. | grain | Tanaka et al. (1986) | 4–670 (53) | 4–312 (65) | 1–3 (13) |
| Canada | grain | Tanaka et al. (1988) | 4–40 (40) | 25–3475 (90) | 2–21 (90) |

[a] Figures in parentheses refer to the percentage of positive samples. [b] Nivalenol. [c] Deoxynivalenol. [d] Zearalenone.

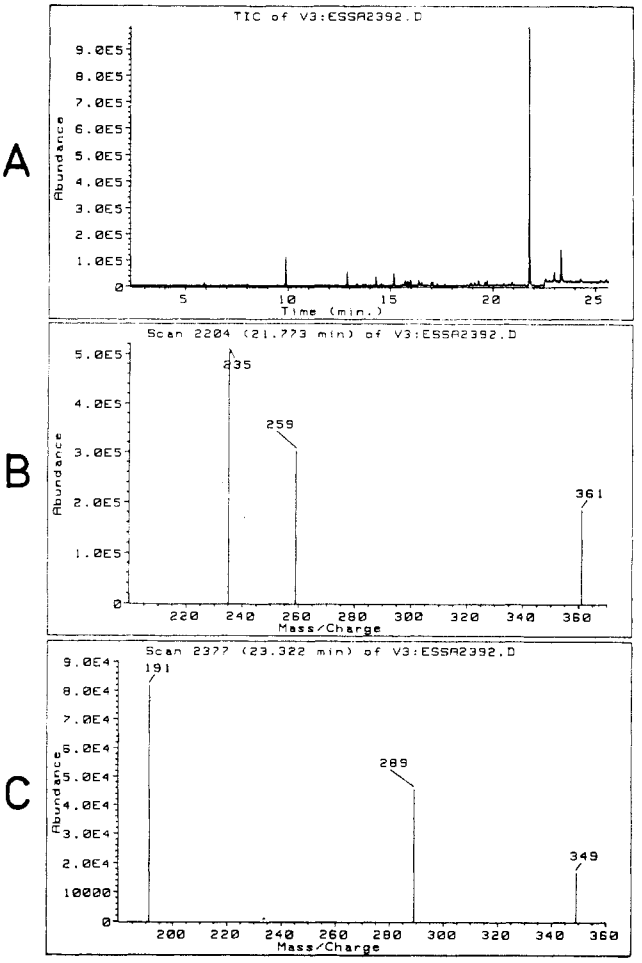


**Figure 5.** (A) SIM total ion chromatogram of a TMS-derivatized sample extract, monitored at ions 235, 259, and 361 (conspecific with TMS-DON) from 0 to 22.5 min and then at ions 191, 289, and 349 (conspecific with TMS-NIV), (B) relative intensity of the ions constituting the peak eluting at 21.77 min, and (C) relative intensity of the ions constituting the peak eluting at 23.32 min.

of DON (210–30 400 ng/g) in selected, heavily infected Polish wheat samples. DON has been a regular contaminant of Canadian wheat grains, where levels of up to 8350 ng/g have been reported from the 1980 winter wheat crop in Ontario (Trenholm et al., 1981, 1983). Scott et al. (1984), however, reported a decline in DON concentrations (59–3450 ng/g) in the 1983 Ontario wheat crop, prior to harvesting. The presence of NIV had not, until recently, been reported in Canadian wheat (Tanaka et al., 1988). The fact that NIV was not previously detected might be attributed to the analytical methods used in the earlier surveys since Scott et al. (1981) stated that NIV was "not carried through our procedures of extraction and clean-up". Since then, both Scott et al. (1986) and Lauren and Greenhalgh (1987) have either improved existing or developed innovative methods and have reported excellent recoveries of NIV, for their respective methods. No reports detailing the natural occurrence of NIV in Canadian wheat using these methods could be found in the literature although Tanaka et al. (1988) recently reported low levels of NIV in Canadian wheat.

Although the natural occurrence of zearalenone in corn- and sorghum-based mixed feeds in South Africa has been reported (Marasas et al., 1977, 1979; Aucock et al., 1980; Thiel et al., 1982a,b; Sydenham et al., 1988), no zearalenone could be detected (at a detection limit of 10 ng/g) in these otherwise highly contaminated wheat samples. These observations are similar to those reported for Canadian (Trenholm et al., 1981) and Polish wheat samples (Visconti et al., 1986) but differ from those reports cited in Table III, where DON and NIV were cocontaminants. With two exceptions, the levels of zearalenone reported were lower than 100 ng/g (Table III). The sensitivities of the methods used by Trenholm et al. (1981) and Visconti et al. (1986) for the determination of zearalenone were, however, relatively poor (i.e., 400 and 500 ng/g, respectively). Therefore, had zearalenone been present in any of the Canadian or Polish wheat samples at levels similar to those generally reported (Table III), it would not have been detected.

In the only previous report of the cooccurrence of NIV and DON in South Africa, levels of 0–1410 and 0–15 800 ng/g, respectively, were found in Transkeian corn (Thiel et al., 1982a). This is, therefore, the first report of the cooccurrence of NIV and DON coupled with the absence of zearalenone in wheat from South Africa.

**Registry No.** DON, 51481-10-8; NIV, 23282-20-4.

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# Chromatographic Profile of Carbohydrates in Commercial Soluble Coffees

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Pure soluble coffee, irrespective of extraction conditions, contains maximum levels of ca. 0.3% total xylose and sucrose, no maltose, and about 2% total glucose. Some commercial soluble coffees have been found to contain one or more of these sugars at higher levels. Elevated levels of total xylose are explained by coextraction with coffee husks/parchment. In this case, the level of free fructose and glucose distinguishes whether unroasted or roasted husks/parchments have been added. Elevated levels of maltose and total glucose indicate the addition of maltodextrins. Elevated levels of sucrose and total glucose show the addition of (caramelized) sugar.

The International Coffee Organization (ICO), an intergovernmental body formed by the coffee producer and consumer countries in close cooperation with the United Nations, defines "green coffee" as "all coffee in the naked bean form before roasting", "roasted coffee" as "green coffee roasted to any degree", and "soluble coffee" as "dried water-soluble solids derived from roasted coffee" (ICO, 1983). Other international bodies, such as the International Standard Organization (ISO), have given equivalent definitions and codified the impurities or "defects" such as wood, sticks, husks, parchment, or whole cherries, which may be present (ISO, 1984). Most countries have stated the maximum amount of defects tolerated in commercial coffee (Jobin, 1982). In applying these standards, visual defect counting is traditionally used to assess the purity of green beans, but this visual approach is obviously inappropriate for soluble coffees.

We have thus developed an analytical method based on screening the carbohydrate profile of instant coffee. The broad base of information obtained not only defines the normal composition of pure soluble coffees coming from a variety of coffee types and processing conditions but also can be used for characterizing the nature of various adulterants or additives occasionally found in these products.

While there have been numerous publications concerning the carbohydrate content in green and roasted coffees, only a few publications give quantitative data, with somewhat conflicting ranges, for the individual carbohydrates of soluble coffee (Streuli, 1970; Thaler, 1957; Pictet, 1975). Arabinose is indicated as the main free sugar (0.4–2.5%), followed by galactose (0.1–1.0%) and mannose (0.2–0.9%). Fructose and glucose were found to be less important (0–0.5%). Only traces of ribose and xylose are reported (Kroplien, 1974). Surprisingly, Trugo and Macrae (1982, 1985) found in a few cases appreciable amounts of free fructose and free glucose. Very little quantitative information is available on the total individual carbohydrates (free sugars plus sugars bound in large molecules): Pictet (1975) found in one sample 16.6% galactose, 12.2% mannose, 4.9% arabinose, and 1.4% glucose; Thaler (1957) quantified by paper chromatography the monosaccharides present in a sulfuric acid hydrolysate and found larger amounts of mannose and galactose and smaller amounts of glucose and arabinose. Only traces of xylose have been found by the various authors.

In the present work, we have established the carbohydrate profiles of many hundreds of soluble coffee samples. This has allowed us to differentiate pure soluble coffee products from adulterated products. It is shown that the values for both free and total sugar contents must be used in order to achieve this and to obtain information on the nature of the adulterants.

## METHODS AND MATERIALS

**HPLC of Sugars.** *Apparatus.* An HPLC system, consisting of a Spectra-Physics SP-1800 (Spectra-Physics Inc., San José, CA), a Kratos URS 051 postcolumn derivatization (Kratos Inc., Ramsey, NJ), a Spectra-Physics SP-8773 XR UV detector, and an HP 1000 integrator, was used. Silica columns (SSMP, Spheri-5 silica 5 μm; Brownlee-Labs, Santa Clara, CA) were used for all analyses. These were prepared with a Supelco saturator with a 18-μm silica phase (Supelco Inc., Bellefonte, CA). The precolumn (Brownlee-Labs SS-GU) was also filled with silica.

*Reagents.* HPLC-grade acetonitrile came from Rowil Chemicals (Shepshed, England). For column preparation, a modified amine was obtained from Prof. Aitzetmüller (Natec, Hamburg, Germany). Tetrazolium Blue for the postcolumn reaction of carbohydrates was supplied by Sigma Chemical Co. (St. Louis, MO). The Carrez solution was prepared by mixing a solution of $K_4Fe(CN)_6 \cdot 3H_2O$, 35.9 g/L in distilled water, and a solution of $ZnSO_4 \cdot 7H_2O$, 71.9 g/L in distilled water (50/50, v/v).

*Preparation of the Column.* After being rinsed with acetonitrile, the precolumn and the column were impregnated with the modified amine solution (Aitzetmüller, 1980). In order to perform an efficient operation, 215 mg of modified amine in 400 mL of acetonitrile and 100 mL of water was recirculated for 15 h at 2 mL/min and at ambient temperature. The mixture was con-

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