Retention of Ergot Alkaloids in Wheat During Processing[1,2]

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ABSTRACT

The retention of six alkaloids in hard red spring wheat infected by ergot *(Claviceps purpurea)* was determined in mill streams and in processed Oriental noodles, pasta, and pan bread. Individual and total alkaloids (ergonovine, ergosine, ergotamine, ergocornine, α-ergokryptine, and ergocristine) were determined by a modification of a previously reported reversed-phase high-performance liquid chromatography procedure. Generally, ergocristine was the predominant alkaloid present, followed by ergotamine. Ergot is more plastic than hard wheat endosperm, hence it flattened during smooth-roll reduction grinding. After milling, ergot alkaloids were concentrated in the late reduction streams and in the shorts derived from the reduction system. Low concentrations of ergot alkaloids were retained in high quality patent flour. Ergot alkaloids were quite stable during end-use processing. Processing of flour into pasta and Oriental noodles had little effect on levels of ergot alkaloids, and a substantial proportion of alkaloids were still present after cooking. However, cooked noodles with alkaline *kansui* contained less alkaloids than either cooked noodles with salt or cooked pasta. Processing flour into pan bread had a minimal effect on alkaloid levels. More alkaloids were present in the crumb than in the outer crust of bread.

Claviceps purpurea (Fr.) Tul. produces pharmacologically potent toxic metabolites (alkaloids) that infect the ovary of cereals, replacing and taking the shape of a kernel as a sclerotium or ergot body. There are no commercially grown cultivars of cereals known to have resistance to *C. purpurea* because infection depends largely on favorable environmental conditions (Lorenz 1979). Without modern cleaning equipment such as gravity tables, removal of ergot from contaminated grains is difficult because ergot bodies take nearly the same size and shape as the kernels of the infected grain (Dexter and Matsuo 1982, Friedman and Dao 1990, Dexter et al 1991).

Ergot is the oldest recorded plant disease, and the toxigenic effects of ergot alkaloids have been recognized for many years. Localized outbreaks of human ergotism still occur (King 1979, Marasas and Nelson 1987, Matossian 1989). Constant vigilance is required to avoid a potential threat to human and animal health.

Ergot alkaloids in ergot-contaminated food and feed are amide or cyclic tripeptide derivatives of lysergic acid. Twelve naturally occurring peptide alkaloids have been isolated and identified (Bianchi et al 1982). These mycotoxins produce symptoms of ergotism in humans and mycotoxicoses in animals (Mantle 1977a,b; Scott and Lawrence 1982; Young and Marquardt 1982; Young et al 1983; Mantle and Willingale 1985; Rotter et al 1985a–c; Marasas and Nelson 1987; Matossian 1989; Rotter et al 1989).

In recognition of the toxicity of ergot, the Canadian Grain Commission has had strict tolerance limits in place for ergot in all cereal grains for many years. For Canada Western Red Spring (CWRS) wheat, the export tolerance limits are 0.01, 0.02, and 0.04% for No. 1 CWRS, No. 2 CWRS, and No. 3 CWRS, respectively, and 0.10% for Canada Western Feed wheat (Canadian Grain Commission 1991).

Few studies on the retention and stability of ergot alkaloids in flour mill streams and processed wheat end-products have been undertaken. Scott et al (1992) reported that low levels of ergot alkaloids were prevalent in Canadian cereal products, with rye flour being the most contaminated food. This study was undertaken to quantitate the retention of individual and total ergot alkaloids in mill streams, bread, Oriental noodles, and pasta derived from ergoty CWRS wheat.

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MATERIALS AND METHODS

Wheat Samples

Wheat samples used for pilot-scale milling were CWRS wheat obtained from the Industry Services Division of the Canadian Grain Commission during the 1992–93 crop year. Thirteen samples weighing ~25 kg each, and representing No. 1, 2, and 3 CWRS wheat were selected based on their ergot content. Following visual inspection, samples were divided into two groups: slightly ergot contaminated (SEC), and heavily ergot contaminated (HEC). The resulting total weight of sample in each group was at least 100 kg.

After individual samples were cleaned in a Carter dockage tester, the cleaned samples in each group were blended together using a Patterson tumble mixer. The resulting composite samples were divided for replicate millings into two portions using a Seedburo Boerner divider.

From each divided SEC and HEC sample, 2-kg subsamples were removed, and sclerotia were hand-picked. A grain inspector verified that the hand-picked samples were free of ergot and that the sclerotia removed were ergot.

An ergot-free CWRS Atlantic cargo sample was used as a control. A rye sample naturally contaminated by ergot was drawn from a grain terminal in Thunder Bay, Canada, and used as a positive check.

Milling

Two lots, 20-kg each of the SEC and HEC CWRS wheat composites were prepared for milling as described by Dexter and Tipples (1987). Each lot was tempered to 16.3% moisture content in a Monarch mortar mixer and rested at 21°C for 18 hr before milling on the Grain Research Laboratory pilot flour mill (Black 1980). The mill flow used in this study is shown in detail in Figure 1.

Mill Products

Flour and by-product streams from each milling were collected individually. Divided flours were prepared by combining portions of appropriate flour streams, selected on the basis of refinement (ash content) and functionality, according to typical Canadian commercial milling practice (Panter 1988).

Millfeed comprised all of the by-product streams: the bran (BR) after bran finishing; the fine bran (FB) and shorts (SH) from the fourth break fine passage (B4F); shorts from the second sizing (S2), fifth middlings (M5), and M6 passages; and shorts flour from the shorts duster (SD). The total yield of millfeed was about 25% of clean wheat, expressed on a constant moisture basis.

Straight-grade flour was prepared from all flour streams produced on the mill; the yield was about 75%. Depending on the wheat, ash content (14% mb) ranged from 0.51 to 0.56%.

A highly refined patent flour corresponding to a typical Canadian household flour was prepared from flour produced by the first and second middlings (M) and first and second sizing (S). The yield of this flour was about 45%; ash content ranged from 0.36 to 0.38%.

A less refined patent flour corresponding to a Canadian bakers' patent flour (yield ~20%, ash content 0.57–0.62%) comprised the flour produced by the first three breaks (B), M3, and M4.

A low-grade clear flour (yield ~10%, ash content 1.19–1.35%) was prepared from the remaining flour streams, which included the fourth break course (B4C) and fourth break fine (B4F), bran finisher (BF), M5, and M6.

Breadbaking

Bread was prepared by the remix baking method (Kilborn and Tipples 1981), a long straight-dough procedure that features an initial fermentation time of 2.75 hr at 30°C. The dough was remixed and given a final proof of 55 min at 30°C. Bread was also prepared by a short straight-dough (no bulk fermentation) procedure. In the short process, the dough was sheeted and panned directly after mixing and given a single proof of 55 min at 30°C. The formula for both procedures (fwb) was: 100 g of flour, 3% compressed yeast, 1% salt, 2.5% sucrose, 15 ppm of potassium bromate, 0.1% ammonium monophosphate, 0.6% malt syrup (6°L) and maximum water while maintaining good dough-handling characteristics. Bread was baked at 225°C for 25 min.

After cooling, bread was cut into two pieces to allow separation of crumb and crust. Crumb and crust were air-dried for two days under subdued light before proceeding with analysis of ergot alkaloids.

Noodle Processing and Cooking

Dried salted noodles that contain 1% NaCl (fwb) and dried Chinese noodles that contain 1% *kansui* (9:1 by weight of Na_2CO_3 to K_2CO_3) were prepared as described by Preston et al (1986) with minor modifications. The flour, salt, or *kansui,* and water (32% absorption on a 14% mb) were mixed for 5 min in a Hobart N50 mixer (Hobart Canada, North York, Ontario) equipped with a paddle blade. The small, crumbly dough pieces were sheeted on a noodle machine (model 150, Ohtake Noodle Machine Mfg., Ltd., Tokyo, Japan). The initial roll gap was set at 3.0 mm. The dough sheet was reduced in thickness gradually by passing through rollers nine times with a final roll gap of 1.2 mm. After the final pass, noodles were cut with a B12 cutter (2.5 mm) and dried for 8 hr in an environment chamber (model 58, Conviron, Winnipeg, Manitoba). Initial drying conditions were 90% rh at 25°C. After 1 hr, temperature was increased to 30°C and rh was gradually decreased to 60% over the remainder of the cycle.

Dried noodles (30 g) were cooked in 300 ml of boiling distilled water for ~13 min. Optimal cooking time was determined as the time when the white core in the strand disappeared following crushing between two glass plates (Dexter and Matsuo 1977). Cooked noodles were drained for 5 min and cooking water was collected. After cooling, drained noodles and cooking water were freeze-dried. Freeze-dried noodles and cooking water were stored in a desiccator at room temperature until analyzed for ergot alkaloids.

Spaghetti Processing and Cooking

Spaghetti was processed in 50-g lots by a micro-spaghetti-making procedure detailed by Matsuo et al (1972). Spaghetti was dried in a constant temperature/humidity cabinet (FR-386 PC-1, Blue M Electric Co., Blue Island, IL) at 39°C (Dexter et al 1981) and at 80°C (Malcolmson et al 1993). Dried spaghetti was cooked for ~14 min. Optimal cooking time and analysis of ergot alkaloids in freeze-dried spaghetti and cooking water were determined as described above for noodles.

Standards

Dry and pure reference standards of six pharmacologically active ergot alkaloids were obtained to make stock solutions for high-performance liquid chromatography (HPLC) and sample fortification. Crystalline ergonovine maleate, ergotamine tartrate, and α-ergokryptine were purchased from Sigma Chemical Co. (St. Louis, MO). Ergocornine maleate, ergocristine, and ergosine were obtained from Sandoz Ltd. (Basel, Switzerland). Dilutions were made with high-purity HPLC-grade methanol except for ergocornine maleate, which was dissolved in pesticide-residue-grade dichloromethane in an ultrasonic bath. Two HPLC working standards were prepared. One consisted of 10 ng/μl of ergonovine and 40 ng/μl of the other alkaloids. The second HPLC working standard was a 10× dilution of the first standard. The sample fortification standard contained 98.6, 403.4, 396.5, 397.4, 401.8, and 399.8 μg/ml of ergonovine, ergosine, ergotamine, ergocornine, α-ergokryptine, and ergocristine, respectively.

Extraction and Cleanup Procedures

Hard red spring wheat samples were ground using a benchtop coffee grinder (Ditting model KFA 900, Elpack Ltd., Toronto, Ontario) set so that a minimum of 90% of the ground grain would pass through a 20-mesh sieve. Bread, noodle, and spaghetti samples were ground before analysis using a hand-held coffee grinder. The extraction methodology described by Scott and Lawrence (1980) was followed with some modifications. A 30-g sample was shaken at high speed for 45 min on a flatbed reciprocating shaker (Eberbach Corp., Ann Arbor, MI) with 20

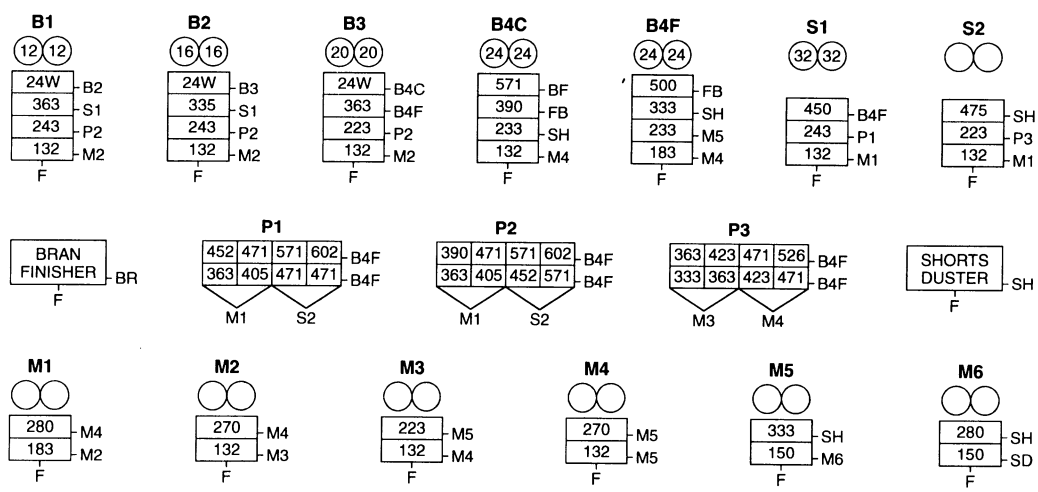

Fig. 1. Grain Research Laboratory pilot mill hard wheat flow. Nitex sieve apertures given in μm; 24-wire aperture 730 μm. Roll fluting expressed as corrugations per inch. B = break (F = fine, C = coarse); S = sizing; M = middling; P = purifer; F = flour; BF = bran finisher; SD = shorts duster; SH = shorts; BR = bran; FB = fine bran.

ml of 4% ammonium hydroxide (ACS-grade) solution and 100 ml of ethyl acetate (distilled in glass) in a 250-ml Teflon centrifuge bottle. While mounted on the reciprocating shaker, the centrifuge bottles were covered with a towel to minimize direct exposure to overhead fluorescent lights. The extract was centrifuged at 3,000 rpm at 4°C (Beckman model GPR, Palo Alto, CA) for 10 min. The organic fraction was filtered through 24-cm Whatman filter paper, and 10 ml of filtrate was dried under nitrogen at 35°C on a heating module (Pierce, Rockford, IL). Sample extracts that were not cleaned up immediately following extraction were stored in a freezer until they could be processed.

Sample extracts were cleaned up on a C_{18} Sep-Pak column (Waters Millipore Corp., Milford, MA) following a procedure based on a modification of a previously reported liquid-solid phase cleanup method for determination of glycoalkaloids in potatoes (Bushway et al 1986). The bonded phase was conditioned by eluting the column with 3 ml of methanol, followed by 5 ml of a buffer solution consisting of 0.02*M* 1-heptanesulfonic acid (sodium salt) in 1% glacial acetic acid (HPLC-grade). All elutions were performed under vacuum with the column connected to a vacuum manifold (Supelco Ltd., Oakville, Ontario). In preparation for cleanup, sample extracts were warmed to room temperature and then reconstituted in 1 ml of methanol with the aid of vortexing for 1 min and sonication for 5 min. A 10-ml aliquot of buffer solution was added to the reconstituted extract, and the mixture was vortexed for 30 sec. The sample extract was poured into a 10-ml gastight syringe barrel that had previously been connected to a conditioned column, and the extract was allowed to elute at a drop-wise rate. Once the sample extract had entered the packing, the column was rinsed with 10 ml of acetonitrile plus nanopure water (1+9, v/v); the column was dried by passing a gentle stream of nitrogen through it for 1 min. All eluates obtained to this point were discarded. The column was then eluted with a mixture of acetonitrile plus buffer solution (1+1, v/v), and exactly 2 ml of the eluate was collected. The cleaned up extract was filtered through a 0.45-μm, 4 mm diameter nylon membrane filter (Gelman Sciences, Ann Arbor, MI) into an amber autosampler vial.

The effectiveness of the analytical method for recovery of the six ergot alkaloids from wheat was checked by analyzing ergot-free samples that had been fortified with a multistandard mixture before extraction. Two fortified 30-g samples of alkaloid-free flour were included in each series of analyses. Samples were fortified with 100 μl of fortification standard to yield fortification concentrations of 0.33, 1.34, 1.32, 1.32, 1.34, and 1.33 ppm of ergonovine, ergosine, ergotamine, ergocornine, α-ergokryptine, and ergocristine, respectively.

Instrumentation and HPLC Conditions

An integrated reversed-phase (RP-HPLC) (Waters Chromatography, Milford, PA) LC module I was employed. The system was equipped with a scanning fluorescence detector (Waters model 470) and a Waters Millenium 2010 Chromatography Manager software system.

Measurement of ergot alkaloids by HPLC was accomplished following a modified version of the procedure described by Scott et al (1992). An isocratic mobile phase was employed using 0.02*M* 1-heptanesulfonic acid in 1% glacial acetic acid (w/v) plus 1% glacial acetic acid in acetonitrile (v/v) (60+40, v/v). All solvents used in the HPLC were filtered through a 0.22-μm (47 mm diameter) nylon membrane filter (Gelman Sciences), placed under vacuum, and degassed by bubbling with helium. Nanopure water was obtained from a Sybron/Barnstead unit (Barnstead/Thermolyne, Inc., Dubuque, IA). A 10-μm C_{18} column (125 A°, 3.9-× 300-mm) from Waters was used. A programmable fluorescence detector was employed with the excitation and emission wavelenghts set at 236 and 416 nm, respectively. Analysis conditions were: flow rate (1 ml/min), injection volume (35 μl), and run time (20 min). Column temperature was controlled at 30°C. All samples were injected into the HPLC in duplicate. New calibration curves were generated for each alkaloid on a daily basis using the integrated peak areas obtained from injection of two different volumes of each HPLC working standard. The minimum reporting limit for each alkaloid was 1 ppb.

Experimental Design

The SEC and HEC composites were each milled in duplicate. Individual mill streams of one milling of the HEC composite were analyzed for ergot alkaloids. Each stream was extracted in duplicate.

The SEC and HEC wheat were ground in duplicate 2-kg lots, and a 30-g portion of each grind was extracted in duplicate. Hand-picked ergot sclerotia from a given sample were composited and ground, and the entire sample was extracted singly.

The clear flours from the HEC millings, and a patent flour from ergot-free CWRS were used for end-product processing. The HEC clear flours were chosen because of the high level of ergot alkaloids, which allowed reliable estimation of ergot alkaloid retention. Before processing, it was verified that no ergot alkaloids were detectable in the patent flour.

The replicate HEC clear flours and the ergot-free patent flour were processed into each end-product in duplicate. The products from each clear flour and products from the patent flour that had been fortified with the ergot alkaloid standards were extracted singly. The rye sample that was naturally contaminated by ergot was extracted and analyzed with each batch of samples as a positive check. Ergot alkaloid concentrations were expressed on a 14% mb.

RESULTS AND DISCUSSION

Ergot Alkaloid Detection

To verify that the extractability of ergot alkaloids was not influenced by the level of ergot alkaloids present, the clear flour from HEC was blended in various proportions from 0 to 100% with an alkaloid-free flour. A linear relationship was found between

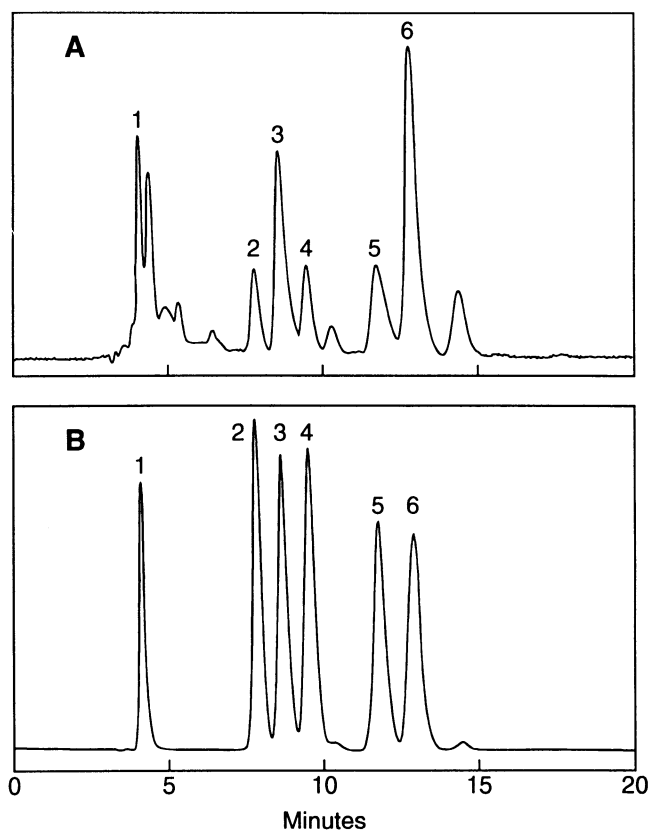

Fig. 2. High-performance liquid chromatography profiles of ergot alkaloids for: clear flour from heavily ergot contaminated (HEC) Canada Western Red Spring wheat **(A)** and reference standards **(B)**. Numbers above peaks indicate ergonovine (1), ergosine (2), ergotamine (3), ergocornine (4), α-ergokryptine (5), and ergocristine (6).

the proportion of clear flour and the measured concentration of individual and total alkaloids (results not shown).

Figure 2 shows chromatographic profiles for the clear flour from HEC and the ergot alkaloid standards. Ergonovine (4.12 ± 0.06 min) was the first alkaloid to elute, followed by ergosine (8.07 ± 0.21 min), ergotamine (8.99 ± 0.26 min), ergocornine (10.02 ± 0.38 min), α-ergokryptine (12.52 ± 0.59 min), and ergocristine (13.83 ± 0.71 min). Standards were prepared monthly to avoid the occurrence of degradation products, most notably for ergocornine and ergocristine (results not shown).

Ergot Alkaloids in Wheat and Sclerotia

Scott et al (1992) reported on the prevalence and concentrations of ergot alkaloids in Canadian grain-based foods. They detected ergot alkaloids in over 50% of products tested. Values that they obtained for positive samples are summarized in Table I. The highest levels were found in triticale and rye products. The principal alkaloids detected in wheat products were ergocristine and ergotamine.

In the current study, ergocristine and ergotamine were also the principal ergot alkaloids detected in both the SEC and HEC wheat samples (Table II). The small sample of sclerotia (ergot bodies) hand-picked from HEC contained a lower proportion of ergocristine than did the wheat (Table II). This is indicative of the variable proportions of ergot alkaloids present in individual ergot bodies. Ergocristine was also the dominant alkaloid present in the rye positive check sample (results not shown). In preliminary studies, when we quantified the ergot alkaloids present in several ergoty rye samples, and in ergoty wheat samples representing all Canadian wheat classes from various locations in Western Canada, we found variable proportions of individual alkaloids, but ergocristine was consistently dominant (results not shown). Young (1981a,b) has reported similar results.

Since the HEC CWRS is near the limit of 0.04% established for No. 3 CWRS, the level of ergot alkaloids in the HEC CWRS is expected to be close to the maximum level of ergot alkaloids that would be found in Canadian CWRS cargoes. Generally, blending of grain unloading at terminals ensures that ergot levels in CWRS cargoes are well below the maximum ergot tolerances.

The possibility that some of the ergot alkaloids present in wheat and wheat products could arise from systemic transfer was discounted by hand-picking the HEC and SEC samples and the diverse set of wheat and rye samples referred to above. In no case were the alkaloids detectable in the grain following hand-picking (results not shown). Therefore, ergot alkaloids detectable in wheat and wheat products are solely due to contamination by sclerotial bodies.

Milling

The Carter dockage tester used in the current study, which simulates a grain separator, was ineffective in removing ergot bodies from the HEC and SEC wheat because ergot bodies have dimensions similar to those of wheat kernels. The cleaning houses of modern commercial mills usually are equipped with devices that remove impurities from wheat on the basis of specific weight. A significant proportion of the ergot would be removed, because ergot bodies have a lower specific weight than do sound wheat kernels (Lippuner 1978, Dexter et al 1991). As a result, the wheat going to the mill would be less contaminated than in the current study, but the by-products from the cleaning house would be heavily contaminated. Concentration of ergot in cleaning by-products would be a concern for mills that combine by-products with millfeed for feed purposes.

As seen in Tables III–V, ergot alkaloids were partitioned in variable concentrations among mill streams in the HEC and LEC wheat samples. In agreement with previous reports (Wolff et al 1983, Baumann et al 1985), the concentration of ergot alkaloids tended to be higher in lower grade (clear) flour and millfeed. However, as seen in Table III, the distribution of ergot among individual mill streams did not correspond directly to the degree of refinement. For example, the broad bran contained a relatively low concentration of alkaloids. Wheat bran separated from the wheat kernel as broad flakes that overtailed the top coarse sieves on the break sifters. The ergot bodies did not form flakes when ground by the corrugated break rolls, and ground ergot was released predominantly into the reduction system along with the intermediate-sized middling particles. Ergot is more plastic than wheat endosperm. As a result, when ergot particles were ground by smooth reduction rolls (S2 and middling rolls) (Fig. 1), they tended to flatten, overtail the top sieves of reduction sifters, and proceeded down the reduction system. As a result, flour streams from the end of the reduction system and shorts derived from the reduction system (including shorts from B4 fine that is fed from the reduction system via purifiers) were the streams where ergot alkaloids were most concentrated. These results are in general agreement with those reported by Shuey et al (1973) for wheat milled on a pilot scale, and by Wolff et al (1983) for wheat milled by a less complex laboratory milling procedure.

The partitioning of ergot alkaloids led to variable concentrations in divide flours for both the HEC and SEC wheat (Tables IV and V). About 25% of the ergot alkaloid present in the wheat before milling was retained in the straight-grade flours. The second patent flours contained slightly higher concentrations. The first patent flour contained the lowest concentration of ergot alkaloids because the grinding passages at the beginning of the reduction system are fed primarily by fine endosperm particles, and by coarser endosperm particles that have been purified. Ergot particles released by the break system were concentrated in the coarser middlings, but were removed during purification. The clear flour had the highest levels of ergot alkaloids, due to the inclusion of late reduction streams.

These results demonstrate the complexity of predicting the concentration of ergot alkaloids in wheat flour. Concentration will increase with extraction rate due to the inclusion of lower grade reduction flours. Concentration will also depend on the milling technique (grinding conditions and mill flow) and the

TABLE I
Summary of Ergot Alkaloids (ng/g) in Canadian Grain Foods from 1985–1991[a]

Ergot Alkaloid	Commodity				
	Rye Flour	Wheat Flour	Bran/Bran Cereal	Triticale Flour	Rye Bread/ Crackers/ Crispbread
Ergonovine	17 (314)	2.9 (16)	4.6 (18)	8.4 (41)	3.4 (67)
Ergosine	31 (718)	3.3 (44)	2.5 (12)	5.9 (30)	9.4 (318)
Ergotamine	63 (1454)	7.5 (64)	6.5 (27)	17 (99)	16 (545)
Ergocornine	22 (415)	2.3 (24)	3.7 (38)	8.0 (53)	4.8 (136)
α-Ergokryptine	25 (271)	3.2 (27)	4.4 (39)	8.0 (82)	2.8 (29)
Ergocristine	81 (847)	12 (73)	15 (51)	39 (241)	9.2 (153)

[a]Scott et al (1992). Mean concentration of alkaloids for positive samples; maximum concentrations are given in parentheses.

TABLE II
Concentration of Alkaloids (ppb) in Ergoty Canada Western Red Spring (CWRS) Wheat and Ergot Sclerotia

Ergot Alkaloid	SEC[a] CWRS	HEC[a] CWRS	Sclerotia[b]	Method Recovery[c] %
Ergonovine	4.0 ± 0.0	15 ± 3.0	6,100	63 ± 5.6
Ergosine	11 ± 6.5	26 ± 6.5	11,000	80 ± 4.6
Ergotamine	23 ± 17	48 ± 7.5	26,000	78 ± 4.4
Ergocornine	1.5 ± 1.5	29 ± 5.5	18,000	76 ± 6.0
α-Ergokryptine	11 ± 10	33 ± 1.0	15,000	75 ± 6.8
Ergocristine	41 ± 30	100 ± 12	34,000	74 ± 6.9
Total	91 ± 29	250 ± 24	110,000	

[a]SEC = slightly ergot contaminated (0.004%); HEC = heavily ergot contaminated (0.03%). Wheat results are mean values (14% mb) of duplicate analyses of duplicate extractions ± deviation about the mean.
[b]Based on a 0.56 g of ergot bodies hand-picked from HEC CWRS wheat sample.
[c]Averages for two fortified samples.

component streams of a given divide flour. The high concentration in the clear flour is of concern because it is used in a variety of edible products. In addition, mills often remove flattened germ as a separate product from the reduction system (Posner 1985). The flattening of ergot during reduction grinding makes it likely that ergot alkaloids would concentrate in the germ.

It is noteworthy that the bran has a much lower concentration of ergot alkaloids than the shorts derived from the reduction system. It is not uncommon for mills to market shorts and bran separately for feed and food purposes.

TABLE III
Concentration of Ergot Alkaloids (ppb) in Individual Mill Streams of Canada Western Red Spring Wheat with 0.030% Ergot (Heavily Ergot Contaminated)[a]

Stream	Ash	Ergonovine	Ergosine	Ergotamine	Ergocornine	α-Ergokryptine	Ergocristine	Total
Red Dog	2.72	80 ± 2.9	310 ± 3.8	760 ± 4.4	480 ± 2.1	910 ± 9.8	2100 ± 73	4700 ± 69
Bread 4 Shorts	3.81	37 ± 13	120 ± 43	390 ± 87	230 ± 75	440 ± 120	1200 ± 260	2400 ± 600
Short Duster	2.78	49 ± 0.8	140 ± 23	340 ± 45	190 ± 71	330 ± 120	750 ± 55	1800 ± 310
Middling 6	1.24	21 ± 1.5	60 ± 3.6	171 ± 1.3	90 ± 4.6	160 ± 9.3	390 ± 27	900 ± 47
Middling 5	1.07	13 ± 0.1	45 ± 0.4	110 ± 0.9	60 ± 2.4	110 ± 3.4	280 ± 7.9	620 ± 15
Middling 4	0.90	6.9 ± 0.8	20 ± 1.6	52 ± 0.4	28 ± 1.5	49 ± 0.8	110 ± 3.9	270 ± 9.1
Break 4 Fine	0.87	6.2 ± 1.4	16 ± 4.4	41 ± 9.7	21 ± 5.2	36 ± 7.6	85 ± 16	200 ± 44
Fine Bran	5.07	4.5 ± 1.8	13 ± 3.9	24 ± 2.2	11 ± 0.9	20 ± 2.2	40 ± 0.6	110 ± 10
Break 4 Coarse	1.16	1.8 ± 0.0	3.9 ± 0.4	11 ± 0.6	5.2 ± 0.1	9.0 ± 0.8	24 ± 2.0	55 ± 1.7
Middling 3	0.51	1.4 ± 0.1	3.1 ± 0.6	8.8 ± 0.8	4.5 ± 0.8	7.6 ± 1.9	19 ± 1.4	44 ± 5.8
Bran Flour	2.83	2.7 ± 0.2	3.7 ± 0.4	8.4 ± 0.3	3.1 ± 0.2	7.2 ± 0.1	19 ± 1.2	44 ± 0.6
Sizing 2	0.49	1.4 ± 0.1	3.0 ± 0.7	7.7 ± 1.1	3.7 ± 0.8	6.6 ± 0.4	15 ± 0.9	37 ± 4.2
Break 3	0.58	1.2 ± 0.3	2.3 ± 1.1	5.9 ± 1.5	2.8 ± 1.1	3.9 ± 1.1	11 ± 2.5	27 ± 7.6
Break 2	0.49	1.0 ± 0.2	1.9 ± 1.5	4.8 ± 2.3	2.1 ± 1.6	3.3 ± 2.3	8.7 ± 4.0	22 ± 12
Bran	6.28	6.3 ± 1.3	TR[b]	5.6 ± 0.8	TR	1.5 ± 0.1	4.5 ± 1.3	19 ± 1.7
Middling 2	0.42	TR	1.1 ± 0.1	3.6 ± 0.4	1.7 ± 0.2	1.9 ± 0.6	7.5 ± 0.8	16 ± 1.6
Sizing 1	0.40	TR	TR	2.9 ± 0.5	1.1 ± 0.7	TR	4.6 ± 1.2	11 ± 3.6
Break 1	0.48	TR	TR	2.6 ± 0.4	1.0 ± 0.5	TR	4.5 ± 0.0	10 ± 1.6
Middling 1	0.34	TR	TR	1.9 ± 0.4	TR	TR	3.6 ± 0.6	7.5 ± 2.8
Method Recovery (%)[c]		67 ± 5.9	84 ± 1.9	82 ± 0.3	81 ± 0.6	81 ± 1.4	80 ± 0.8	

[a]Mill stream values (14% mb) are means of duplicate analyses of duplicate extracts ± deviation about the mean for two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding and inclusion of trace level amounts.
[b]Detected at a concentration <1.0 ppb.
[c]Averages for two fortified samples.

TABLE IV
Concentration of Ergot Alkaloids (ppb) in Divide Flours and Millfeed of Heavily Ergot Contaminated Canada Western Red Spring Wheat (0.03% Ergot)[a]

Ergot Alkaloid	First Patent	Second Patent	Straight Grade	Clear	Millfeed	Method Recovery[b] %
Ergonovine	ND[c]	1.9 ± 0.2	1.3 ± 0.4	12 ± 1.5	17 ± 0.3	59 ± 0.9
Ergosine	ND	4.2 ± 0.1	2.6 ± 0.8	34 ± 0.4	38 ± 11	76 ± 0.6
Ergotamine	TR[d]	14 ± 0.1	10 ± 2.9	91 ± 11	82 ± 19	74 ± 1.7
Ergocornine	ND	5.9 ± 0.3	3.9 ± 0.7	48 ± 1.2	51 ± 21	71 ± 0.3
α-Ergokryptine	ND	12 ± 1.4	8.4 ± 1.5	82 ± 3.6	84 ± 31	69 ± 0.8
Ergocristine	2.3 ± 1.1	32 ± 2.0	23 ± 3.8	200 ± 16	180 ± 60	68 ± 0.5
Total	2.6 ± 1.3	69 ± 4.0	49 ± 10	470 ± 30	460 ± 140	

[a]Mean values (14% mb) ± deviation about the mean for duplicate analyses of duplicate extracts from two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding and inclusion of trace level amounts.
[b]Averages for two fortified samples.
[c]Not detected.
[d]Detected at a concentration <1.0 ppb.

TABLE V
Concentration of Ergot Alkaloids (ppb) in Divide Flours and Millfeed of Slightly Ergot Contaminated (SEC) Canada Western Red Spring Wheat (0.004% Ergot)[a]

Ergot Alkaloid	First Patent	Second Patent	Straight Grade	Clear	Millfeed	Method Recovery[b]
Ergonovine	ND[c]	TR[d]	TR	2.7 ± 0.3	12 ± 1.2	63 ± 5.6
Ergosine	ND	TR	TR	6.6 ± 0.9	14 ± 2.4	80 ± 4.6
Ergotamine	ND	1.2 ± 1.0	1.2 ± 1.0	19 ± 5.0	25 ± 7.5	78 ± 4.4
Ergocornine	ND	ND	ND	4.9 ± 3.4	4.5 ± 3.9	76 ± 6.0
α-Ergokryptine	ND	TR	TR	16 ± 1.4	15 ± 1.9	75 ± 6.8
Ergocristine	ND	5.2 ± 0.6	4.7 ± 1.4	50 ± 14	53 ± 17	74 ± 6.9
Total	ND	7.3 ± 0.6	6.6 ± 3.3	99 ± 24	120 ± 30	

[a]Mean values (14% mb) ± deviation about the mean for duplicate analyses of duplicate extracts from two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding and inclusion of trace level amounts.
[b]Averages for two fortified samples.
[c]Not detected.
[d]Detected at a concentration <1.0 ppb.

Bread

Fermentation time did not affect the stability of ergot alkaloids during baking (Table VI). Whether fermented for a short period or a long period, bread baked from the HEC clear flour exhibited no loss of alkaloids in the crumb. However, alkaloid losses of 25–55% were evident in the crust. Lower concentrations of ergot alkaloids in the crust are likely due to higher crust temperatures during baking.

Some previous reports have indicated ergot alkaloid losses of 50% or more occur during baking of a variety of products (Baumann et al 1985; Wolff et al 1985, 1988; Friedman and Dao 1990, Scott and Lawrence 1982). Wolff et al (1985) reported that the extent of ergot alkaloid denaturation during baking is related to the baking temperature and the location within the baked product. As discussed by Scott and Lawrence (1982), there is a thermal gradient in bread during baking, and the lower heat transfer to the interior of the loaf contributes to the greater stability of ergot alkaloids in the crumb than in the crust. However, other factors such as the initial alkaloid levels in the flour, baking procedure and formula, dough thickness, interaction of alkaloids with dough components during baking, and the relative susceptibilities of individual alkaloids to heat damage, could influence the extent of alkaloid degradation (Friedman and Dao 1990).

In this study, the quantitation of ergonovine in the crust was masked by a large, interfering peak. Hence, no concentrations of ergonovine in the crust are reported. A study conducted by Scott et al (1992) had the same difficulty in quantifying ergonovine due to the complete masking of the peak by coextractives. All of the other alkaloids exhibited comparable (~75%) retention in the crust.

Oriental Noodles

Lower levels of ergot alkaloids were detected in the uncooked noodles (Table VII) than in the parent HEC clear flour (Table IV). The loss of total alkaloids ranged from 11–46% and from 35–42% for uncooked noodles processed with salt and *kansui,* respectively. In view of the stability of alkaloids in baked bread, which has a higher water activity and is subjected to more thermal stress than noodles, it seems unlikely that the lower alkaloid levels detected in the noodles are due to denaturation during processing. It may be that alkaloid extraction was incomplete because of the coarser particle size of the ground uncooked noodles compared to the flour. Insufficient material remained to verify whether this was the case.

Cooked noodles contained slightly lower levels of total alkaloids than the corresponding uncooked noodles. Losses in cooked *kansui* noodles (60–75% compared to those of flour) were greater than those in cooked salted noodles (47–49%). Relatively low levels of alkaloids were found in the cooking water from both noodles. Individual alkaloids exhibited comparable stability during noodle processing and cooking.

These results indicate that alkaloids were retained in uncooked and cooked noodles, and were not leached out into the cooking water to a large extent. It appears that alkaloids are relatively stable in noodles after cooking and are strongly bound to the noodle matrix. Matossian (1989) reported that toxicity of alkaloids was stable for 18 months, and that alkaloids do not break down during baking at low heat or after boiling for 3 hr.

In the present study, cooked noodles containing *kansui* retained less alkaloids than cooked noodles containing salt. This observation could be due to the alkaline condition in the *kansui* noodles, promoting structural modifications of the alkaloids. Ware et al (1986) found that mixtures of ergot alkaloid isomers were stable even at room temperature.

Spaghetti

As in the uncooked noodles, lower levels of ergot alkaloids were detected in the uncooked spaghetti (Table VIII) than in the parent HEC clear flour (Table IV); this was possibly due to the coarseness of the ground uncooked spaghetti. Regardless, the loss of ergot alkaloids in uncooked spaghetti was only about 20–42%, and the drying cycle temperature did not affect alkaloid retention.

Loss of alkaloids in the cooked spaghetti ranged from 42–79%, with higher losses being evident in the spaghetti dried at high temperature (Table VIII). The level of alkaloids detected in the cooking water was low regardless of the drying cycle used. Individual alkaloids exhibited similar stability during spaghetti processing and cooking.

These results are consistent with the results for Oriental noodles discussed previously (Table VII). Although cooking caused a reduction in alkaloid levels in the pasta, it was not due exclusively to extensive leaching into the cooking water. However, as reported by Dexter and Matsuo (1982), when spaghetti heavily contaminated with ergot was cooked, the cooking water became dark, which is indicative of some leaching of ergot components into the cooking water.

TABLE VI
Concentration of Ergot Alkaloids (ppb) in Remix Bread Processed from Heavily Ergot Contaminated Clear Flour[a]

Ergot Alkaloid	Short Process		Long Process		Method Recovery (%)[b]
	Crumb	Crust	Crumb	Crust	
Ergonovine	11 ± 0.6	ND[c]	12 ± 3.2	ND	69 ± 0.5
Ergosine	34 ± 12	23 ± 12	36 ± 8.6	25 ± 3.9	85 ± 0.5
Ergotamine	86 ± 14	63 ± 14	96 ± 16	62 ± 8.9	89 ± 0.4
Ergocornine	40 ± 9.1	24 ± 2.6	45 ± 18	29 ± 13	83 ± 0.6
α-Ergokryptine	50 ± 12	30 ± 9.8	55 ± 10	34 ± 5.4	75 ± 0.4
Ergocristine	160 ± 25	100 ± 21	180 ± 27	120 ± 22	70 ± 0.1
Total	390 ± 54	240 ± 60	420 ± 66	270 ± 46	

[a] Mean values (14% mb) ± deviation about the mean from replicate bakes of clear flour from two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding and inclusion of trace level amounts.
[b] Averages for two fortified samples.
[c] Interfering peak present and ergonovine not detected.

TABLE VII
Concentration of Ergot Alkaloids (ppb) in Oriental Noodles Processed with Salt and *Kansui* from Heavily Ergot Contaminated Clear Flour[a]

Ergot Alkaloid	Uncooked Noodles		Cooked Noodles		Cooking Water		Method Recovery (%)[b]
	Salt	*Kansui*	Salt	*Kansui*	Salt	*Kansui*	
Ergonovine	11 ± 3.8	7.7 ± 5.2	8.0 ± 4.1	3.8 ± 0.8	4.6 ± 3.2	9.3 ± 2.9	66 ± 19
Ergosine	23 ± 3.7	26 ± 1.7	17 ± 3.8	15 ± 10	5.1 ± 2.0	1.6 ± 4.0	84 ± 11
Ergotamine	60 ± 11	54 ± 3.7	39 ± 29	22 ± 6.5	11 ± 0.9	ND[c]	83 ± 12
Ergocornine	35 ± 6.1	22 ± 7.8	23 ± 4.6	13 ± 5.2	6.2 ± 1.3	6.1 ± 3.2	92 ± 13
α-Ergokryptine	42 ± 11	34 ± 14	21 ± 6.5	17 ± 8.2	3.5 ± 1.8	3.0 ± 1.5	85 ± 10
Ergocristine	120 ± 32	110 ± 12	95 ± 21	61 ± 67	10 ± 1.5	7.4 ± 1.2	83 ± 9.5
Total	290 ± 67	250 ± 41	210 ± 63	130 ± 95	41 ± 6.8	28 ± 4.8	

[a] Mean values (14% mb) ± deviation about the mean from replicate processings of clear flour from two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding.
[b] Averages for two fortified samples.
[c] Not detected.

TABLE VIII
Concentration of Ergot Alkaloids (ppb) in Spaghetti Processed from Heavily Ergot Contaminated Clear Flour and Dried at 39 and 80°C[a]

Ergot Alkaloid	Uncooked Spaghetti		Cooked Spaghetti		Cooking Water		Method Recovery (%)[b]
	39°C	80°C	39°C	80°C	39°C	80°C	
Ergonovine	34 ± 4.7	7.2 ± 1.8	10 ± 7.9	8.8 ± 10	2.1 ± 1.4	3.8 ± 0.9	65 ± 6.2
Ergosine	25 ± 6.4	26 ± 2.8	19 ± 5.8	13 ± 7.4	3.5 ± 1.0	3.1 ± 0.5	88 ± 4.7
Ergotamine	57 ± 4.9	58 ± 8.5	43 ± 17	28 ± 12	8.4 ± 1.0	7.9 ± 0.4	86 ± 8.8
Ergocornine	24 ± 7.5	25 ± 6.4	17 ± 1.5	12 ± 0.6	3.4 ± 0.4	2.8 ± 1.5	86 ± 4.7
α-Ergokryptine	29 ± 7.5	28 ± 1.6	22 ± 7.5	16 ± 3.4	3.7 ± 0.9	2.3 ± 0.9	85 ± 4.7
Ergocristine	110 ± 8.9	111 ± 18	64 ± 21	42 ± 17	8.1 ± 1.5	6.3 ± 1.3	83 ± 4.5
Total	280 ± 25	260 ± 30	180 ± 44	120 ± 52	30 ± 5.6	26 ± 1.4	

[a]Mean values (14% mb) ± deviation about the mean from replicate processings of clear flour from two millings. Totals may not equal the sum of concentrations of the individual alkaloids due to rounding.
[b]Averages for two fortified samples.

CONCLUSIONS

Ergocristine was the predominant ergot alkaloid found in wheat grown in Western Canada. Cleaning of wheat by the Carter dockage tester did not remove ergot bodies effectively. For efficient removal of ergot alkaloids, cleaning equipment that separates impurities on the basis of specific gravity is required.

Alkaloids were stable during milling and were partitioned over a broad range of concentrations into the millfeed and the flour streams. Ergot is more plastic than hard wheat endosperm, and flattens during smooth roll reduction grinding. As a result, the greatest levels of ergot alkaloids were detected in the late reduction flours and in the shorts derived from the reduction system. Relatively low levels were detected in the bran and break flours. The lowest levels were found in the early reduction flours.

Individual alkaloids appeared equally stable during processing into bread, pasta, and Oriental noodles. Ergot alkaloids were very stable during bread processing and baking. Some alkaloids were lost during cooking of Oriental noodles and spaghetti, but total retention in the cooked products consistently exceeded 25% of that in the flour. The broad range of alkaloid concentrations observed in the millfeed and flour streams from ergoty wheat, and the different stability of the ergot alkaloids depending on the final end-product produced, underline the complexities of establishing safe tolerance limits for ergot.

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