Losses of Ergot Alkaloids during Making of Bread and Pancakes

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Studies were carried out to determine losses of six ergot alkaloids naturally occurring in whole wheat flour, rye flour, and triticale pancake mix after baking into bread or, with the latter flour, making into pancakes. Bread was also baked with spiked ergot-free flour. Estimations were made by gradient liquid chromatography (LC) with fluorescence detection, and mean overall alkaloid recoveries from flour and bread were 73–79% (peak height measurement). Mean losses on processing, although not determinable in some cases because of interferences, ranged up to 100% during baking of whole wheat bread, up to 85% during baking of rye bread, and up to 74% during making of triticale pancakes.

Cereal grain and grasses may become contaminated with ergot sclerotia formed by *Claviceps* species. These sclerotia contain variable amounts of ergot alkaloids, in particular ergometrine, ergotamine, ergosine, ergocornine, α-ergokryptine, and ergocristine, which are the major pharmacologically active alkaloids of wheat and rye ergots (Hofmann, 1964; Lorenz, 1979; Mantle et al., 1977; Young, 1981a,b). Toxic effects in animals and humans include action on the uterus, the heart and circulation, the central nervous system, the endocrine system, and reproductive processes (Berde and Schild, 1978). Cleaning and milling grain remove most of the ergot that might end up in flour (Shuey et al., 1973). However, recent research has shown that commercial wheat and rye flour contain ergocristine, ergotamine, and other alkaloids in small concentrations (<100 μg/kg) (Scott and Lawrence, 1980). Chemical determination of individual alkaloids integrated with toxicological data is a more reliable indication of any potential hazard in using ergot-contaminated feed or food than counting ergot bodies or particles (Young, 1979). An expanded survey of flour and grain foods for ergot alkaloids is needed. Prior to this, a study of the effect on the alkaloids of processing flour into food such as bread would indicate where analytical effort should be concentrated. It is known that ergot alkaloids are only moderately stable when heated in aqueous solution; ergotamine, for example, undergoes conversion to the inactive isomer ergotaminine (Bethke et al., 1976; Güven and Güneri, 1972). Moreover, alkaloid concentrations in ground ergot sclerotia decrease on storage (Bano et al., 1976). The stability studies described here were carried out by application of gradient liquid chromatography (LC) for the determination of alkaloids in whole wheat flour, rye flour, a pancake mix containing triticale flour, and breads or pancakes made from them.

EXPERIMENTAL SECTION

Baking Experiments. Flours and pancake mix naturally contaminated with ergot alkaloids were used, except for an experiment with ergot-free flour containing no detectable ergot alkaloids which was baked with and without addition of 5 μg/kg ergometrine and 20 μg/kg other alkaloids. Whole-grain wheat bread and all rye flour bread were baked in an oven (Precision Scientific Co., Chicago, IL) at 180 °C for 1 h, essentially following recipes given by Rombauer and Becker (1962) adjusted for using ~300 g of flour and substituting water for milk. Proportions of whole wheat flour to all-purpose enriched flour were 2 to 1. Triticale pancakes were made from 113 g of triticale pancake mix (containing triticale flour and wheat flour), two medium eggs, 0.25 L of water, and ca. 6 g of baking powder; the mixture was heated in an electric frying pan at 204 °C for 2 min. Cooked products were broken up, dried at room temperature overnight, weighed, ground in a Waring Blendor (wheat bread and the pancakes) or Retsch centrifugal grinding mill (rye bread), and mixed before analysis.

Extraction and Cleanup. Duplicate 25- or 50-g subsamples were extracted by the method described by Scott and Lawrence (1980) with the following slight modifications: (1) extracted flour was rinsed with ca. 3 mL of methanol following the methylene chloride washes; (2) the initial extract residue was dissolved in 40 mL of ether, and up to 3 mL of methanol was added after the second extraction with 0.5 N hydrochloric acid if needed to clear emulsions; (3) ca. 10 mL of 28% aqueous ammonia solution was used to raise the pH of the hexane-washed acid extract to 10.0.

Ergot Alkaloids. These were received as gifts (see Acknowledgment) or purchased from Sigma Chemical Co., St. Louis, MO 63178 (ergometrine; α-ergokryptine). Isomerizations were carried out as described previously (Scott and Lawrence, 1980).

Liquid Chromatography. The apparatus used for gradient LC consisted of two Altex pumps (Model 110A), solvent programmer (Altex Model 410), and solvent mixer. Sample and standard solutions (0.125 μg of ergometrine/mL, 0.5 μg each of ergosine, ergotamine, and ergocornine per mL, 0.375 μg of α-ergokryptine/mL, 0.75 μg of ergocristine/mL, and appropriate dilutions, in methanol) were injected onto a 4.6 mm i.d. × 25 cm column packed with 5-μm LiChrosorb RP-8 or Spheri-5 RP-8 by using a Valco Universal Inlet (25-μL loop) or Rheodyne Model 7125 syringe loading sample injector (20-μL loop). Mobile-phase composition was programmed from 28 or 29 to 43% acetonitrile in 200 mg/L aqueous ammonium carbonate solution or 0.1 M KH_2PO_4–0.1 M NaOH (50:44) buffer adjusted to pH 7.6 or 7.7 or (with Spheri-5 RP-8) from 32 to 45% acetonitrile in 0.025 M KH_2PO_4–0.025 M NaOH buffer (pH 7.7) by using exponent setting 5 for 10 min and then holding until elution of ergocristine. Although not done in this study, it is wise to use a silica gel saturator precolumn before the injector to enhance the lifetime of the analytical column at these alkaline pHs. The flow rate was 1.1–1.5 mL/min. Acetonitrile was previously filtered through 0.5-μm Fluoropore type FH filters (Millipore), and aqueous solutions were previously filtered through 0.45 μm MF-Millipore type HA filters. The alkaloids were detected by fluorescence measured with a Schoeffel FS-970 variable-wavelength fluorometer at an

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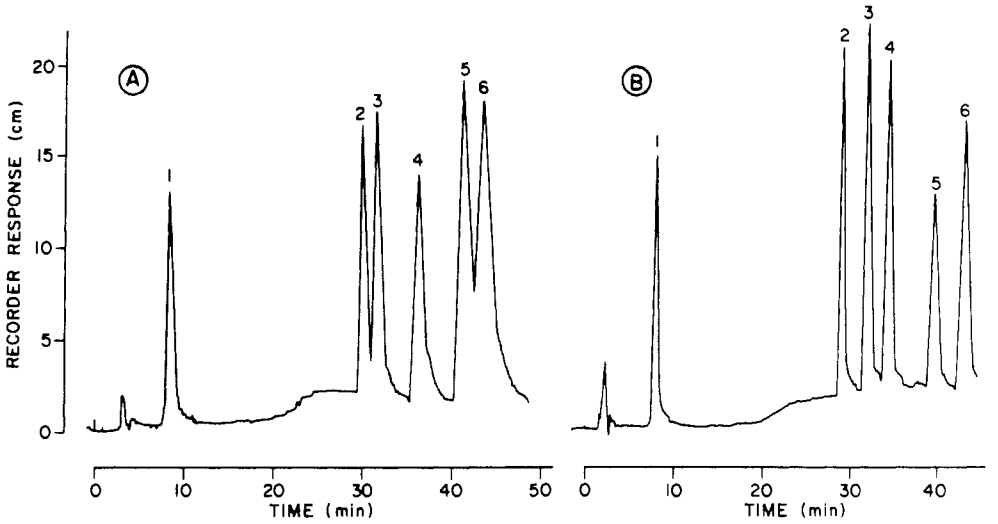

Figure 1. LC of ergot alkaloids. (A) On the LiChrosorb RP-8 column; 2.5 ng of ergometrine (peak 1), 10 ng of ergosine (peak 2), 10 ng of ergotamine (peak 3), 10 ng of ergocornine (peak 4), 20 ng of α-ergokryptine (peak 5), and 20 ng of ergocristine (peak 6); gradient elution 29–43% acetonitrile in 0.1 M KH_2PO_4–0.1 M NaOH (50:44) buffer adjusted to pH 7.7; flow rate 1.1 mL/min; range setting 0.05 μA full scale. (B) On the Spheri-5 RP-8 (5 μm) column; 2.5 ng of ergometrine (peak 1), 10 ng of ergosine (peak 2), 10 ng of ergotamine (peak 3), 10 ng of ergocornine (peak 4), 7.5 ng of α-ergokryptine (peak 5), and 15 ng of ergocristine (peak 6); gradient elution 32–45% acetonitrile in 0.025 M KH_2PO_4–0.025 M NaOH (50:44) buffer adjusted to pH 7.7; flow rate 1.4 mL/min; range setting 0.05 μA full scale.

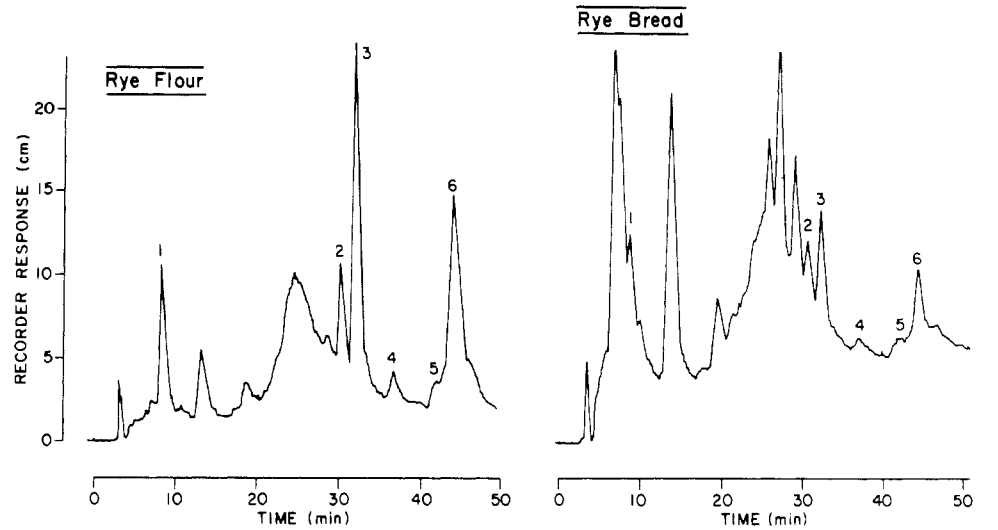

Figure 2. LC of extracts of rye flour 2 (250 mg equiv injected) and corresponding dried rye bread (500 mg equiv, from 467 mg of rye flour) containing respectively concentrations estimated (by peak height) of 6.2 and 1.6 μg/kg ergometrine (peak 1), 16 and 4.5 μg/kg ergosine (peak 2), 48 and 7.8 μg/kg ergotamine (peak 3), 7.8 and 2.0 μg/kg ergocornine (peak 4), 0 and 2.4 (interference) μg/kg α-ergokryptine (peak 5), and 53 and 12 μg/kg ergocristine (peak 6); LC conditions similar to those in Figure 1A.

Table I. Recoveries of Ergot Alkaloids Added to Flour and Ground Bread[a]

	ergometrine		ergosine		ergotamine		ergocornine		α-ergokryptine		ergocristine	
	peak ht	integr	peak ht	integr	peak ht	integr	peak ht	integr	peak ht	integr	peak ht	integr
mean	73.1	80.0	76.8	75	78.9	77.0	74.9	75.9	77.2	78.6	78.0	73.0
n	26	17	18	18	28	18	18	18	28	18	28	18
std. dev.	19.1	35.5	15.0	14.5	10.2	13.8	14.4	18.6	12.7	17.3	12.7	15.7
coeff var., %	26.1	44.3	19.5	19.3	12.9	17.9	19.2	24.5	16.5	22.0	16.3	21.5

[a] Samples used and maximum number of experiments (in parentheses) were enriched flour (8), ergot-free flour (10), low alkaloid (2.7 μg/kg ergocristine) rye flour (4), whole wheat bread (4), and rye bread (2) at spiking levels of 2.5–15 μg/kg (ergometrine) and 5–30 μg/kg (other alkaloids). Corrections were made for contribution of blank, if applicable.

excitation wavelength of 235 nm (emission filter KV 370). Determination of alkaloids was by peak height and (for recovery experiments also) peak area (Spectra-Physics minigrator) and comparison with standard curves. The attenuation was 0.05-μA full scale. Between runs, the LC column was flushed with acetonitrile for ca. 10 min and then reequilibrated for 10 min.

RESULTS AND DISCUSSION

The major change in methodology since publication of our previous paper on analysis of ergot alkaloids in flour (Scott and Lawrence, 1980) has been the introduction of

gradient LC, which shortens the time required for the determinative step. We have used two reverse-phase columns in this work and observed a marked improvement in resolution of α-ergokryptine from ergocristine on the Spheri-5 RP-8 column compared to LiChrosorb RP-8 (Figure 1). The main differences between the two phases appear to be that they use spherical and irregular microparticulate silica gels, respectively, as base materials and the former phase is also endcapped (Majors, 1980). Mean overall recoveries from grain foods for the six alkaloids under investigation were 73–79% (peak height measurement) with a lower precision for ergometrine compared to the other alkaloids (Table I). Since peak height measurement gave estimates of precision that were equal to or better than those obtained by integration (Table I), it was subsequently chosen for the actual sample analyses in the baking and cooking experiments (Tables II–IV). Agreement between replicate determinations of individual alkaloids in the naturally contaminated flours and processed products was generally good: only in four analyses, involving ergotamine (experiment 3, Table II) and ergocornine (experiments 1 and 2, Table II) in whole wheat flour and ergocristine in triticale pancake mix (Spheri-5, Table IV), were observed coefficients of variation greater than 30%, and in 86% of the remaining positive analyses they were less than 20%. Some problems with interferences were noted, depending on the reverse-phase column and the product analyzed. With the LiChrosorb RP-8 column, there were interferences for ergometrine and α-ergokryptine in rye bread and a generally high background in the ergosine region for extracts of rye flour, triticale pancake mix, and the corresponding rye bread and pancake (Figures 2 and 3). The Spheri-5 RP-8 column resulted in several interferences. Interferences for ergometrine in rye bread and two of three whole wheat breads made determination impossible; ergotamine was only partially resolved from an interference in one of the whole wheat flours and one whole wheat bread and not resolved in two other breads (Figure 4), and there was even a small interference in bread made from ergot alkaloid free flour; with the whole wheat and rye flours and rye bread, there was a small interference following α-ergokryptine and not quite separated from it; while rye flour, rye bread, and triticale pancake (Figure 5) showed a similar interference just after ergocristine (see Tables II–IV). By comparison with LiChrosorb RP-8, on which the isomeric alkaloids, except ergometrinine, all eluted after ergocristine (Scott and Lawrence, 1980), ergocristine and ergotaminine were not resolved on the Spheri-5 RP-8 column (Table V), resulting in probable overestimination of ergocristine. Apart from the whole wheat flour 1 (Table II) and a rye flour, which contained respectively about 16 and 13% ergotaminine relative to ergocristine (by area), we have not determined ergotaminine in foods. However, there was fairly good agreement between the two columns for ergocristine analyses in whole wheat flour (experiments 1 and 2, Table II), rye flour (experiments 2 and 3, Table III), and triticale flour (Table IV).

Despite these difficulties, after considering results with the two RP-8 columns, it was possible to make estimates of the losses upon baking or cooking of most of the ergot alkaloids occurring naturally in samples of whole wheat flour, rye flour, and triticale pancake mix (Tables II, III, and IV). Losses were greatest in baking of whole wheat bread from whole wheat flour and enriched flour (Table II), up to 100% for four of the five alkaloids where estimation was possible; ergocristine losses were at least 69%. Complete destruction of alkaloids was also observed in

Table II. Losses of Ergot Alkaloids during Baking of Whole Wheat Bread

alkaloid	est concn, enriched flour, μg/kg[a,b]	expt 1			expt 2			expt 3			expt 4		
		est concn, μg/kg[a]			est concn, μg/kg[a]			est concn, μg/kg			est concn, μg/kg[a]		
		whole wheat flour 1[c]	bread (dried)[b]	mean loss, %[d]	whole wheat flour 1[b]	bread (dried)[b]	mean loss, %[d]	whole wheat flour 2[b,e]	bread (dried)[a,b]	mean loss, %[d]	ergot alkaloid free flour, spiked[b,f]	bread (dried)[b]	mean loss, %[d]
ergometrine	0.4	3.5	I[g]		2.2	I		2.0	ND[h]	100	3.6	ND	100
ergosine	0.5	6.7	ND	100	2.3	ND	100	1.9	ND	100	15.2	ND	100
ergotamine	1.5	8.6	4.3[i]	[26]	9.1[i]	7.1 (I)		7.4	8.4 (I)		14.3	1.0 (I)	[92]
ergocornine	0.6	7.3	ND	100	6.3	ND	100	3.5	ND	100	15.5	ND	100
α-ergokryptine	0.8	8.3	ND	100	6.2[i]	1.0	87	2.8[i]	0.8	59	14.4	ND	100
ergocristine	3.1[j,k]	23	4.6[k]	[69]	19[i]	2.1[k]	[83]	19[k]	5.4[k]	[59]	13.2	ND	100

[a] Means of duplicate determinations (peak height measurements). [b] Spheri-5 RP-8 column. [c] LiChrosorb RP-8 column. [d] Calculated on the basis of alkaloid weight loss from weight originally in flour. Loss in brackets is unreliable (see footnotes g, i, k, and l). [e] Means of triplicate determinations (peak height measurements). [f] 5 μg/kg ergometrine plus 20 μg/kg other alkaloids. [g] I, interference. [h] ND, not detected. [i] Only partial resolution of peak from interference. [j] Previous analyses 2.8 and 3.0 μg/kg with the LiChrosorb RP-8 column. [k] May include some ergotamine. [l] Includes equivalent to 3 μg/kg ergotaminine.

Table III. Losses of Ergot Alkaloids during Baking of Rye Bread

alkaloid	expt 1 est concn, μg/kg[a,b] flour 1	expt 1 bread (dried)	expt 1 loss, %[d]	expt 2 est concn, μg/kg[a,b] flour 2	expt 2 bread (dried)	expt 2 loss, %[d]	expt 3 est concn, μg/kg[a,c] flour 2	expt 3 bread (dried)	expt 3 loss, %[d]
ergometrine	2.7	2.3 (I)[e]		5.3	1.7	66	6.1	I	
ergosine				14	4.4	67	14[f]	2.9	77
ergotamine	8.6	3.6	54	43	7.5	81	51	7.3	85
ergocornine				6.9	2.0	69	6.4	1.2	81
α-ergokryptine	2.2	1.9 (I)		ND[g]	2.4 (I)		7.2[h]	1.4[h]	[80][i]
ergocristine	13	5.9	50	46	10	76	58[h,j]	9.7[h,j]	[82][i]

[a] Means of duplicate determinations (peak height measurements). [b] LiChrosorb RP-8 column. [c] Spheri-5 RP-8 column. [d] Calculated on the basis of alkaloid weight loss from weight originally in flour. [e] Interference. [f] Single determination only. [g] ND, not detected. [h] Only partial resolution of peak from interference. [i] Unreliable (see footnotes *h* and *j*). [j] Measured as ergocristine but not separated from any ergotaminine present.

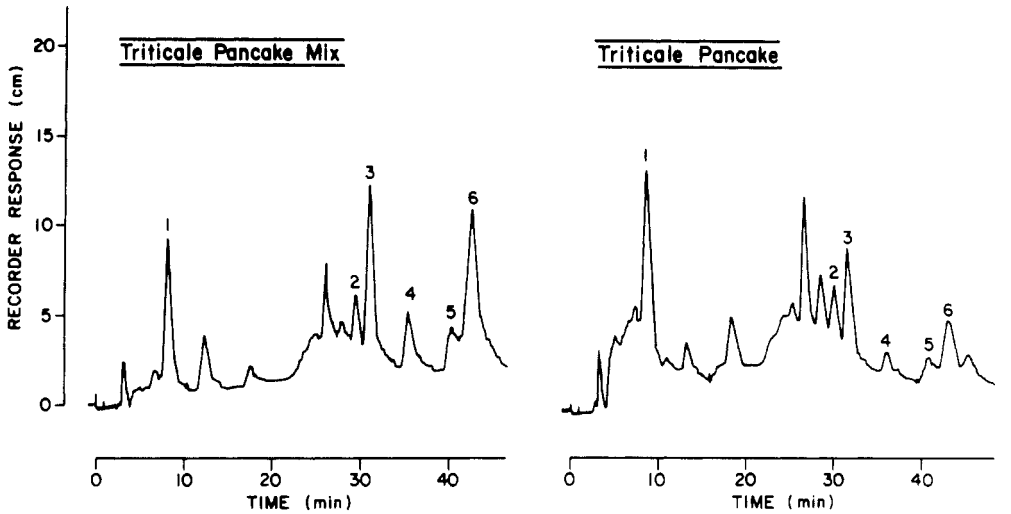

Figure 3. LC of extracts of triticale pancake mix (100 mg equiv injected) and dried pancake made from this mix (167 mg equiv of pancake injected, from 146 mg of mix). For these analyses, the samples were estimated (by peak height measurements) to contain respectively 16 and 11 μg/kg ergometrine (peak 1), 20 and 11 μg/kg ergosine (peak 2), 59 and 20 μg/kg ergotamine (peak 3), 29 and 6.6 μg/kg ergocornine (peak 4), 29 and 9.0 μg/kg α-ergokryptine (peak 5), and 108 and 23 μg/kg ergocristine (peak 6); LC conditions as in Figure 1A.

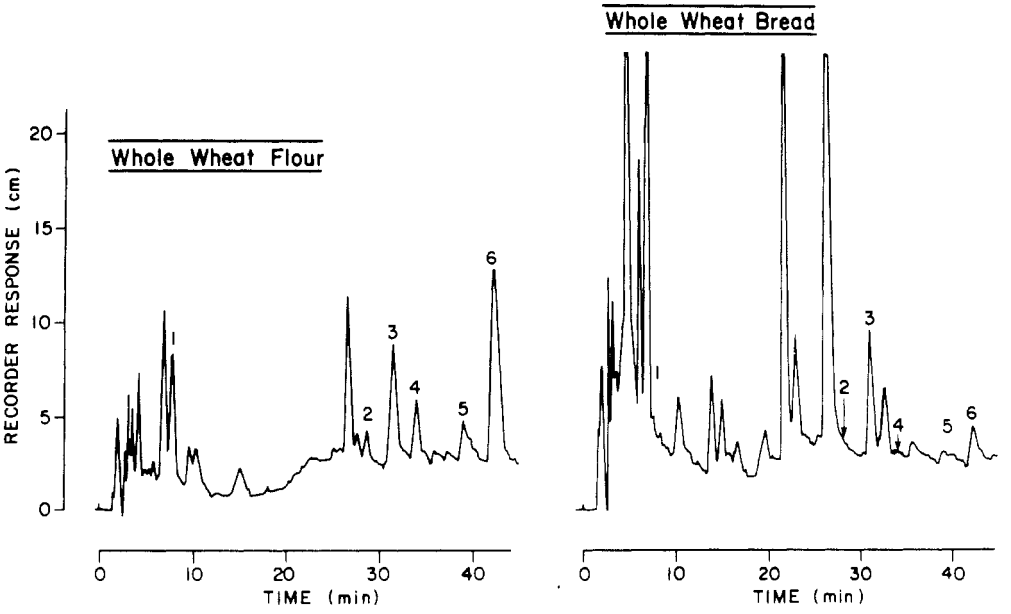

Figure 4. LC of extracts of whole wheat flour 2 (500 mg equiv injected) and dried bread (500 mg equiv injected) made from this flour (307 mg) and all-purpose enriched flour (154 mg). For these analyses, the samples were estimated (by peak height measurement) to contain respectively 1.9 and 0 μg/kg ergometrine (peak 1), 2.1 and 0 μg/kg ergosine (peak 2), 6.7 and 7.3 (interference) μg/kg ergotamine (peak 3), 4.0 and 0 μg/kg ergocornine (peak 4), 3.2 and 0.8 μg/kg α-ergokryptine (peak 5), and 22 and 4.7 μg/kg ergocristine (+ergotaminine) (peak 6); LC conditions as in Figure 1B.

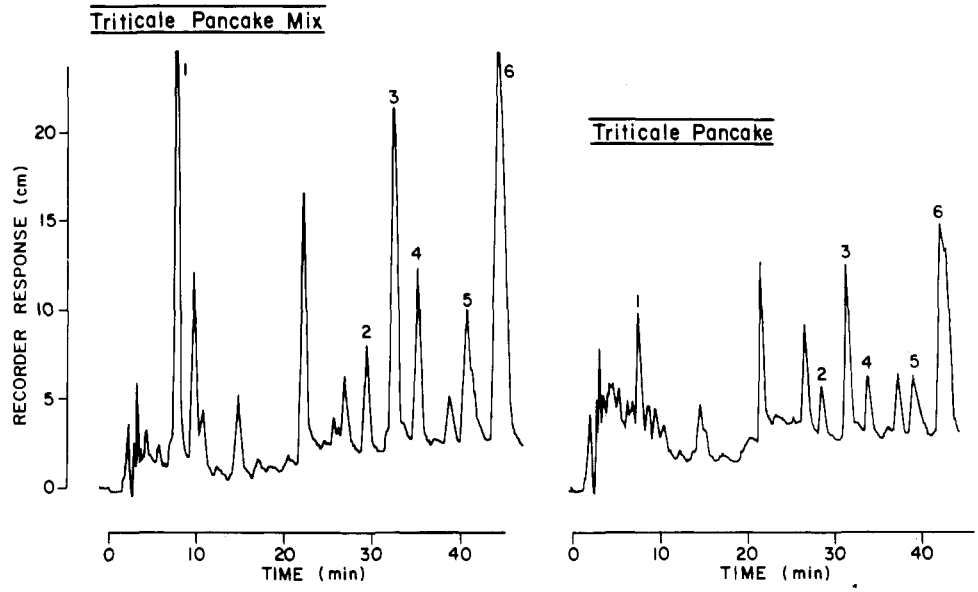

Figure 5. LC of extracts of same triticale pancake mix as in Figure 3 (250 mg equiv injected) and corresponding dried pancake made from this mix (250 mg equiv injected, from 196 mg of mix). For these analyses, the samples were estimated (by peak height measurement) to contain respectively 19 and 5.6 μg/kg ergometrine (peak 1), 13 and 6.8 μg/kg ergosine (peak 2), 40 and 20 μg/kg ergotamine (peak 3), 22 and 8.0 μg/kg ergocornine (peak 4), 20 and 9.8 μg/kg α-ergokryptine (peak 5), and 96 and 51 μg/kg ergocristine (+ergotaminine) (peak 6); LC conditions as in Figure 1B.

Table IV. Losses of Ergot Alkaloids during Making of Triticale Pancakes

	LiChrosorb LC column			Spheri-5 LC column				
	est concn, μg/kg[a]			est concn, μg/kg				
					dried pancake[a]		loss, %[b]	
alkaloid	triticale mix	dried pancake	loss, %[b]	triticale mix[c]	expt 1	expt 2	1	2
ergometrine	15	10	25	16	3.6	5.8	74	54
ergosine	18	10	34	13	5.2	6.1	54	41
ergotamine	53	22	53	41	20	19	45	40
ergocornine	26	7.2	69	20	6.0	8.0	65	48
α-ergokryptine	29	9.4	63	20	7.4	9.1	58	43
ergocristine	99	26	70	88[d]	29[d,e]	45[d,e]	[62][f]	[35][f]

[a] Means of duplicate determinations (peak height measurements). [b] Calculated on the basis of alkaloid weight loss from weight originally in mix. [c] Means of four determinations (peak height measurements). [d] Measured as ergocristine but may include some ergotaminine. [e] Only partial resolution of peak from interference. [f] Unreliable (see footnote *d*).

Table V. Retention Times of Isomeric Ergot Alkaloids on Spheri-5 RP-8

	retention time, min[a]	
alkaloid pair	-ine	-inine
ergometr(in)ine	8.6	16.7
ergosin(in)ine	30.0	38.3
ergotam(in)ine	33.5	44.9
ergocorn(in)ine	35.1	49.0
α-ergokrypt(in)ine	40.6	60.8
ergocrist(in)ine	44.9	70.3

[a] Gradient LC; 32-43% acetonitrile in phosphate buffer, pH 7.7; flow rate 1.34 mL/min.

baking bread from ergot alkaloid free flour spiked with 5 μg/kg ergometrine and 20 μg/kg other alkaloids. Destruction of ergot alkaloids was less during baking of rye bread, probably because of lower heat transfer to the interior of the loaf, and best estimates ranged from 66 to 85% (Table III); thus, some alkaloids could be expected to be found in commercial rye bread. The shorter processing time entailed in making pancakes still resulted in 53–74% greatest loss of individual alkaloids (Table IV), indicating the sensitivity of these compounds to heat in a food matrix. However, in view of the high levels of ergot alkaloids that can be found in triticale products, appreciable concentrations may still remain after cooking.

In conclusion, in order to obtain data on intake of ergot alkaloids by humans based on surveys of grain foods, analytical resources should be given primarily to those foods that have not undergone severe heat processing. The effects of home cooking must also be taken into account.

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Polyphenols of Sorghum Grain, Their Changes during Malting, and Their Inhibitory Nature

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An automated system for the detection of tannins is described. It uses the precipitation of bovine serum albumin (BSA), and this, in tandem with an automated detection of phenolic groups, has been used for the rapid investigation of four sorghum varieties. Two of the varieties were bird resistant and were distinguishable from the non-bird-resistant grains by the presence of a tannin fraction that precipitates protein. The noninhibiting fraction (F_1) of all four varieties contained a large number of varied phenolics. During malting the roots and shoots developed a large complement of F_1 polyphenols and the properties of the tannins changed. The ability of the grain to resist mold growth is related to polyphenol content; resistance was ascribed to the physical barrier set up by the tannin-containing testa. Formaldehyde treatment of whole grain reduced both the number of available phenolic hydroxyl groups in the tannin and its ability to precipitate BSA.

Polyphenols are a large and important group of secondary metabolites found in higher plants. One group of polyphenols comprises the tannins, and besides giving the usual phenolic reactions, they also have the ability to precipitate proteins. It is these compounds that are found in the testae of certain varieties of grain sorghum, thus making them bird-resistant.

These tannins can inactivate enzymes required during the brewing of sorghum beer (Daiber, 1975) and also reduce the nutritional quality of the grain. Because of this it is important to develop a better understanding of tannins. The monomeric building blocks of tannins have been established (Gupta and Haslam, 1978), and a sequence for their biosynthesis has been proposed (Gupta and Haslam, 1979).

Work has recently been reported on the extraction of the polyphenols from bird-resistant sorghums and their separation into several fractions (Kaluza et al., 1980). Aqueous acetone (70%) was found to be a good solvent for sorghum polyphenols and it did not extract proteins. For separation of the polyphenols Sepharose CL-6B was used. It separated the polyphenols into three fractions; the first fraction contained low molecular weight polyphenols which did not precipitate protein while the other two fractions contained tannins of different molecular weights and they did precipitate protein.

The present study uses these techniques to compare the polyphenols of two bird-resistant sorghum varieties with those of two non-bird-resistant varieties. While working with the bird-resistant grains we observed that their polyphenolic patterns changed during malting. One purpose of this study was to observe this phenomenon more closely. Additionally, we have tried to overcome the inhibitory nature of the tannins by treating a bird-resistant grain with formaldehyde.

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EXPERIMENTAL PROCEDURES

Four varieties of sorghum grain, SSK2, NK300, DC36, and Breytenbach Red, were collected as described before (Kaluza et al., 1980). The first two were bird-resistant varieties, with SSK2 having the higher tannin content. The last two are not bird resistant but represent marketing classes known in South Africa as KM and KR, respectively. Additional SSK2 grain was (a) treated with formaldehyde and (b) malted and the phenolic pattern examined.

Extraction and Separation of Polyphenols. The polyphenols were extracted with aqueous acetone and separated into the major fractions (F_1, F_2, and F_3) by using an acetone gradient on Sepharose CL-6B columns (Kaluza et al., 1980). Usually, 50 g of ground grain was extracted for each column run. Two gradients were applied sequentially: (1) H_2O–H_2O–80% v/v aqueous MeOH (250:250:300 mL); (2) 80% aqueous MeOH–25% v/v aqueous Me_2CO–50% Me_2CO (280:250:264 mL). This solvent was found to be the best of many tried, and it separated the polyphenols of sorghum into a fraction containing the noninhibitory polyphenols and two fractions containing the inhibitory tannins.

A dimethylformamide (DMF) elution was also used during the study of the effect of formaldehyde on polyphenols. It consisted of two parts: (1) H_2O–H_2O–80% aqueous MeOH (250:250:300 mL); (2) 80% aqueous MeOH–20% v/v aqueous DMF–40% aqueous DMF (250:209:211 mL). DMF was sometimes used in preference to acetone because acetone interferes slightly with the automated phenolic detection system (Kaluza et al., 1980).

Automated Detection of Tannins by BSA Precipitates. We have been able to show in this study that if the pH is constant at pH 4.8 and ionic strength is 0.1, then the precipitation of BSA is proportional to the amount of

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