JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Article

Ammonization of the *R*- and *S*-Epimers of Ergot Alkaloids to Assess Detoxification Potential

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Cite This: *J. Agric. Food Chem.* 2022, 70, 8931−8941

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ABSTRACT: Detoxification of ergot-contaminated feed by ammonia would be a practical application, given that ammonia is routinely used in the agriculture industry. To assess the effects of ammonia on ergot alkaloids, natural ergot-contaminated wheat was ammoniated. The total concentration of ergot alkaloids (*R*- and *S*-epimers) decreased after exposure to ammonia (8−29%). Separately, the total *R*-epimers decreased in concentration (40−66%), whereas the total *S*-epimers increased (21−81%). Specific ergot alkaloids demonstrated degradation and/or epimerization after exposure to ammonia, potentially associated with structural differences, and influenced the total concentrations observed. Ammonization of ergot standards resulted in potential degradation products and epimerization, supporting the above results. The use of ultrahigh-performance liquid chromatography−tandem mass spectrometry provides an updated assessment of the detoxification potential of ammonia for ergot alkaloids and the quantification of the *S*-epimers. Ammonia alters the *R*- and *S*-epimers of ergot alkaloids, which may lead to a potential practical detoxification process of ergot-contaminated feed.

KEYWORDS: *mass spectrometry, contamination, ammonia, feed, safety*

INTRODUCTION

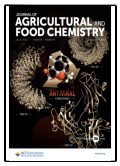
Ergot sclerotia in grains intended for human and animal food and feed are monitored. An assessment can determine if the contaminated grains are suitable for human consumption or are downgraded to animal feed. In the past decade, the occurrence of ergot alkaloids in Canada and the United States has increased.[1] Recently, 25−50% of wheat in Western Canada was downgraded associated with ergot contamination.[2] Grain is downgraded depending on the amount of ergot sclerotia contamination. In Canada, grain with the highest grade contains less than 0.04% mass of sclerotia/mass of grain, whereas feed grain contains less than 0.10%, for multiple wheat types.[3] A concentration range of 2000−9000 μg ergot alkaloid/kg feed for livestock in Canada has been established depending on the animal species.[4] Safety guidelines establish concentrations of ergot alkaloids in feed for the protection of animal health. Billions of dollars are lost annually due to ergot-contaminated grain because of the impact on livestock productivity[5] and financial penalties related to grain quality.

The removal of ergot sclerotia and detoxification of ergot alkaloids is a priority in agriculture. Simply, ergot sclerotia can be separated by size, density, and color from healthy grain kernels.[6] However, ergot alkaloids can be detected within the dust and fines in contaminated grain after the removal of ergot sclerotia.[7] Additional methods for physical reduction of ergot alkaloids include heat and ultraviolet light,[8] food processing techniques,[9−11] and the addition of binders.[12] Biological methods of detoxification of ergot alkaloids are limited; however, some fungi and bacteria have been shown to decrease the concentration of ergot alkaloids.[1] Chemical approaches for the detoxification of ergot alkaloids have included the use of chlorine, ozone, sulfur dioxide, bleach, hydrogen peroxide, and ammonia.[5]

Exposure of ergot-contaminated matrices to ammonia has been investigated. Ergot-contaminated hay was ammoniated and fed to steers.[13] The steers had lower body temperatures and mitigated toxic manifestations compared to the steers fed nonammoniated ergot-contaminated hay. The authors, however, did not assess the concentration of ergot alkaloids within the hay after ammonization. In related research, ergovaline, an ergot alkaloid common in infected tall fescue, had a significantly reduced concentration of 54% with exposure to 2% ammonia for 1 week.[14] The authors reported that the reduction in the ergot alkaloid was not affected by the concentration of ammonia; however, a time-dependent decrease in concentration over several weeks was observed. Likewise, another study observed a decrease in ergot alkaloid concentrations exposed to ammonia for 6 weeks; however, the decrease was not statistically significant.[15] Comparably, another study showed that ammonia treatment did not affect the total concentration of ergot alkaloids.[6] Overall, ammonization of ergot may be influenced by multiple factors, producing various results.

Ammonia is utilized in the agriculture industry for multiple applications. Low-quality forage is ammoniated to increase its nutritional value for livestock.[16] It is routinely utilized when

Received: March 4, 2022
Revised: June 27, 2022
Accepted: June 28, 2022
Published: July 13, 2022



https://doi.org/10.1021/acs.jafc.2c01583
J. Agric. Food Chem. 2022, 70, 8931−8941

feed sources are limited.[17] Digestibility and crude protein content increase when feed is exposed to ammonia.[16] Readily-accessible ammonia would allow for practical applications for the detoxification of ergot-contaminated feed while increasing the nutritional value.

Studies assessing the effects of ammonia on ergot alkaloids only consider the C-8-(*R*)-isomers of ergot alkaloids and not the C-8-(*S*)-isomers for quantification.[5,13,14] A rotation at the chiral carbon 8 adjacent to the carbon 9−10 double bond of the chemical structure defines the epimer of the ergot alkaloid (Figure 1). The C-8-(*R*)-isomers, which will be referred to as

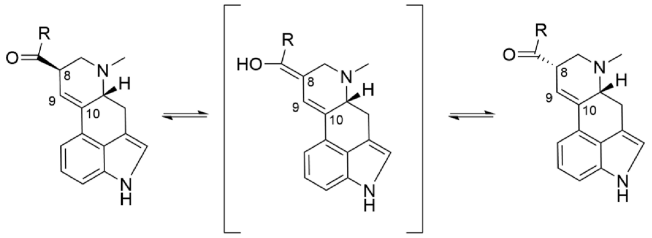


Figure 1. Reaction scheme of the epimerization process. From left to right: [C-8-(*R*)-isomer (*R*-epimer), intermediate structure, C-8-(*S*)-isomer (*S*-epimer)].

the *R*-epimers, are designated with a "-ine" suffix, whereas the C-8-(*S*)-isomers, which will be referred to as the *S*-epimers, are designated with an "inine" suffix. The *S*-epimers of ergot alkaloids constitute a large proportion of the total concentration of ergot alkaloids.[18] Potentially, studies assessing the *R*-epimers of ergot alkaloids after exposure to ammonia, may misrepresent the overall impact of ammonization on ergot alkaloids. Effects of ammonization could potentially be unrecognized without *S*-epimer quantification. The different configurations of ergot alkaloids can interconvert to one another, with the *R*-epimer[19] and the *S*-epimer[20,21] causing toxic effects. Historically, the *R*-epimer is thought to be more toxic than the *S*-epimer.[1] It is critical to evaluate the degree of ammonization of both configuration forms to assess the detoxification potential.

Further investigation into the ammonization of ergot alkaloids to assess potential detoxification is required. The objective of this study was to examine the effects of ammonia on the concentration of the total, total *R*, and total *S*-epimers of ergot alkaloids and assess if individual ergot alkaloids respond differently after exposure to ammonia. A preliminary study was conducted to provide further insight into the ammonia−epimer degradation process. The use of ultrahigh-performance liquid chromatography−tandem mass spectrometry (UHPLC−MS/MS) allows for the detection and quantification of configuration differences using robust and sensitive data. Results from this study could potentially lead to a practical solution to detoxify ergot-contaminated feed.

MATERIALS AND METHODS

Samples. Six independent ergot-contaminated hard red spring wheat samples were obtained from the Canadian Feed Research Centre (North Battleford, Canada). Visually, the six samples contained a very high quantity of ergot sclerotia. The samples had a concentration range of 377,164−787,310 μg/kg of total ergot alkaloids. Therefore, each sample was diluted with clean wheat as described previously.[22] Samples were ground using a UDY Cyclone Sample Mill (Fort Collins, USA, Model #3010−060, 1 mm mesh) and analyzed using UHPLC−MS/MS. After analysis, each sample had a similar starting concentration, with a sample average of 841 μg/kg and a range of 756−943 μg/kg of total ergot alkaloids. The total ergot alkaloid concentration includes 12 ergot epimers, namely, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergocornine, ergocorninine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, and ergotaminine.

Ammonization. Samples were ammoniated following a previous method with modifications.[23] A small glass Petri dish was placed on the bottom of a 38 oz Anchor Hocking sealable jar (Canadian Tire, Saskatoon, Canada). The Petri dish contained 2 mL of ammonium hydroxide (NH_4OH) (Honeywell, Fisher Scientific) diluted with deionized water. The NH_4OH stock solution contained approximately 30% NH_3, and dilutions were made to achieve two separate concentrations of NH_3 with a desired final concentration of 2 or 5% NH_3 per weight of grain. The 2 and 5% NH_3 concentrations were made by combining 0.33 and 0.83 mL, respectively, of NH_4OH, with 1.67 and 1.17 mL, respectively, of deionized water. A segment of Everbilt Garden black plastic mesh fence, with a 1/2 in. square mesh opening (Home Depot, Saskatoon, Canada), was placed on top of the Petri dish within the jar. A weigh boat containing 5 g of ground ergot-contaminated wheat was placed on top of the plastic mesh. The weigh boat was hexagonal with a diameter of 5−5.5 cm. The ground grain was smoothed out with firm plastic across the surface area of the weigh boat. The depth of the grain sample in the weigh boat was approximately 0.2 cm. This was conducted for all samples to obtain uniformity of depth and similar surface area to be exposed. Ammonia vapors, from ammonium hydroxide, provided ammonia (NH_3)

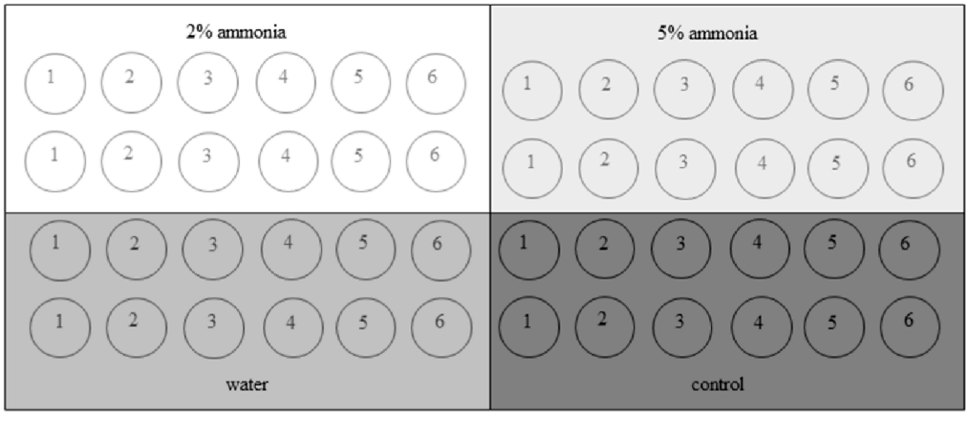


Figure 2. Experimental design setup. Subsamples from the six (1−6) natural ergot-contaminated samples were placed in jars (circles), in two replicates, within each group (2% ammonia, 5% ammonia, water, and control). This design was set up independently for 1, 2, and 3 week incubation periods with subsamples from the six samples replaced each time.

https://doi.org/10.1021/acs.jafc.2c01583
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exposure to the grain. The jar lid was closed and sealed for the incubation period at room temperature. All jars were covered with black plastic to deter light exposure during the incubation period.

Experimental Design. The effects of two ammonia concentrations (2 and 5%) on ergot epimers after incubation for 1, 2, or 3 weeks were assessed. The ammonia concentrations and times were chosen based on practical[24] and literature[14] recommendations. In total, 48 jars were placed into four groups of 12 jars each. Each group had either 2% ammonia concentration, 5% ammonia concentration, water, or nothing (control group), pipetted into the Petri dish. Within each of the four groups, subsamples from the six ($n = 6$) independent ground ergot-contaminated samples were placed into the jars with two replicates of each subsample (Figure 2). Replicates of subsamples were utilized to minimize the effect of sampling variability. Averages of replicate values were utilized in all analyses. All 48 jars were sealed, covered with black plastic, and incubated for 1 week. At the end of the incubation period, the jar lids were opened, and the ammoniated grain samples were removed and placed in a fume hood for 45 min to allow for excess ammonia to escape. Forceps were used to break up the grain into very small pieces to aerate the grain which clumped due to moisture. Each grain sample was then put into a 50 mL metal-free centrifuge tube for the extraction and analysis of ergot epimers. This entire procedure was repeated separately, with new subsamples from each of the six samples replaced each time, and incubated for either 2 or 3 weeks.

Ergot Epimer Extraction and Analysis. The extraction and analysis of the ammoniated ergot-contaminated samples followed a protocol previously described.[22] In brief, 20 mL of the extraction solvent containing acetonitrile/water (80:20) was added to the 50 mL centrifuge tubes. The samples were mixed for 1 h, followed by centrifugation for 10 min at 3500 rpm. The supernatant of each sample was filtered through a 0.45 μm polytetrafluoroethylene filter. A 160 μL aliquot of the filtered supernatant and 40 μL of the internal standard, deuterated lysergic acid diethylamide, were added to amber glass vials and dried down with nitrogen. The dried samples were reconstituted in 50:50 methanol/water, transferred to amber vials with 200 μL inserts, centrifuged, and placed into the autosampler for UHPLC−MS/MS analysis. The UHPLC system used was a ThermoFisher Scientific Vanquish with a 2.1 mm ID filter cartridge and a Hypersil GOLD C18 Selectivity column (ThermoFisher Scientific, Waltham, Massachusetts, USA), coupled to a ThermoFisher TSQ Altis MS/MS (triple quadrupole MS/MS). An injection of 2 μL was followed by a gradient of mobile phase A (water with 0.1% formic acid) and mobile phase B (methanol with 0.1% formic acid). Electrospray ionization in positive mode and selective reaction monitoring were utilized for the detection and quantification of the 12 ergot epimers.

Statistical Analysis. Subsamples, from samples 1−6, were exposed throughout the experiment to either 2% NH_3, 5% NH_3, or control at either 1, 2, or 3 weeks. The water group was removed from the analysis due to mold growth on all the samples. Estimates from the subsamples were determined for each ammonia concentration/time. To determine whether ammonia concentration and/or time affects the concentration of total, total *R*, or total *S*-epimers, the estimates of subsamples from the six samples exposed to either 2% NH_3, 5% NH_3, or control at either 1, 2, or 3 weeks were compared.

The statistical analyses were performed using SPSS 23 (IBM SPSS Statistics for Windows, version 23, IBM Corp., Armonk, NY). Generalized estimating equations (GEE) was the statistical analysis utilized with an identity link function, robust errors, and an unstructured correlation matrix to account for repeated measures of subsamples from the six samples exposed to each ammonia concentration and time. Estimates from subsamples were used in the analysis to compare groups (ammonia-exposed and control) at the three time periods. Normality of data was tested utilizing a one-sample Kolmogorov−Smirnov test, even though normality is not an assumption of the GEE. A normal distribution was considered at $P > 0.05$. In the presence of a significant interaction between the effects of ammonia concentration and time on mean epimer concentration, GEE was used each time to assess the effects of ammonia. A statistically significant difference was considered at $P < 0.05$ for all analyses. A significant effect of ammonia resulted in a multiple pairwise comparison between ammonia-exposed groups and the control group, with a sequential Sidak correction, at each time period. This was executed separately for the total concentration of ergot epimers (*R*- + *S*-epimers), total *R*-epimer concentration, and total *S*-epimer concentration. Comparison of ergot epimer concentrations between ammonia-exposed groups at each of the time periods is discussed as a mean percent increase or decrease compared to their control group. The results for the effects of ammonia on each individual ergot alkaloid (*R*- and *S*-epimer pair) are presented descriptively.

Ammonia−Epimer Degradation Products. A preliminary study was conducted to assess the potential chemical reaction between ammonia and ergot epimers, and *R*- and *S*-epimer standards were exposed to ammonia vapors and NH_4OH directly following a previous method with modifications.[23] Ergocristine and ergocristinine (Romer Labs, Tulln Austria) were used as the representative *R*- and *S*-epimer. Ergocristine and ergocristinine were dried down separately at a concentration of 1 μg/kg in an HPLC amber vial under a stream of nitrogen. Following the ammonization technique, the amber vials were placed inside the jars with 2 mL of NH_4OH with approximately 30% NH_3 in the glass Petri dish. The NH_3 concentration was utilized to ensure that a reaction was observed. Jars were sealed, and the samples were incubated for 2 and 3 weeks. Black plastic was used to cover the jars. At 2 and 3 weeks, the lids of the jars were opened in the fume hood for 15 min to allow excess ammonia to escape. The samples were reconstituted in 1 mL of methanol/water (50:50) for analysis. Control samples of ergocristine and ergocristinine were executed in the same manner; however, no NH_4OH was added to the glass Petri dish. To assess the full degradation of ergot epimers, after the dry-down of ergocristine and ergocristinine separately, the ergocristine and ergocristinine standards were reconstituted in 100 μL of NH_4OH, with a concentration of 1 μg/kg for each epimer. Subsequently, NH_4OH was allowed to evaporate in the fume hood. Once dried, the samples were reconstituted in 100 μL of methanol/water (50:50) for analysis. The analysis for all the samples was carried out as mentioned previously; however, these samples were analyzed in quadrupole one full-scan mode with a range of m/z 560−660. This range was chosen based on the range utilized by Borràs-Vallverdú et al. (2020),[23] with modifications for the different molecular weights of the compounds of interest. The adducts of interest would hypothetically be in this range. Since this section of the study is preliminary, the results will be reported descriptively.

RESULTS

Ammonization of Ergot-Contaminated Grains. The concentrations of the total ergot epimers, within the evaluated groups of control, 2% ammonia, and 5% ammonia at 1, 2, and 3 weeks, were normally distributed ($P > 0.05$). The total concentration of ergot epimers in naturally contaminated grains was altered with exposure to ammonia and time. There was a significant interaction between ammonia concentration and time effects on the total epimer concentration (GEE, Wald chi-square = 147.82, df = 4, $P < 0.001$). In weeks 1, 2, and 3, there was a significant effect on the mean total epimer concentration related to ammonia concentration (GEE, Wald chi-square = 15.74, 41.16, and 144.17, respectively, df = 2, $P < 0.001$ for all weeks). After 1 week of incubation in 2% ammonia, an 8% decrease in the total concentration of ergot epimers was observed in the ammonia-exposed group compared to the control group. No statistically significant difference in the total ergot concentration was observed after 1 week of incubation in 5% ammonia (multiple pairwise comparisons with sequential Sidak correction, $P = 0.061$). In weeks 2 and 3, there was a decrease in the total concentration of epimers exposed to 2 and 5% ammonia (multiple pairwise

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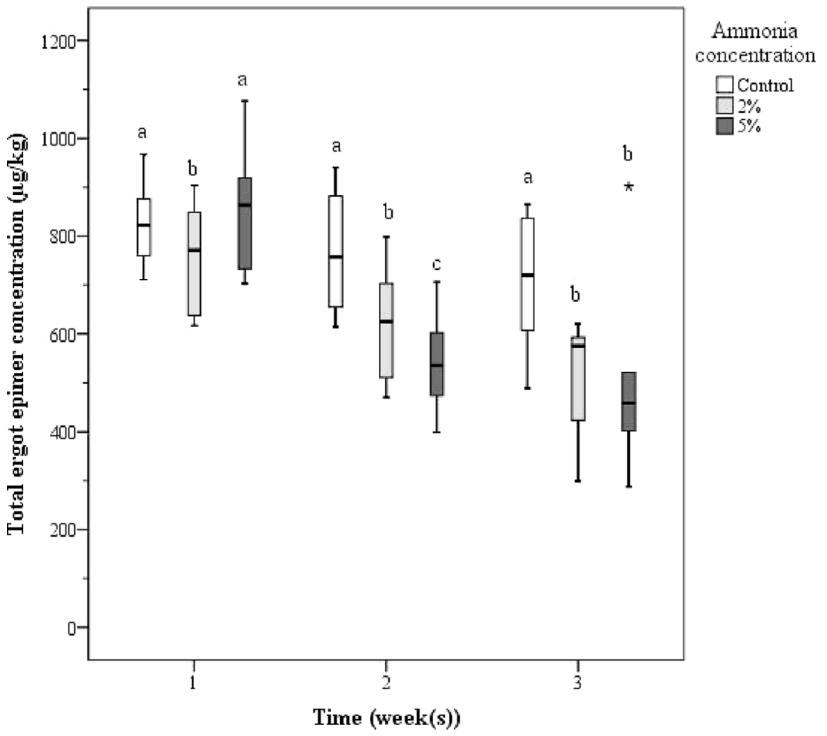

Figure 3. Total ergot epimer concentration at each time period and ammonia exposure. Total concentration (μg/kg) of all 12 epimers in natural ergot-contaminated wheat after exposure to each concentration of ammonia (%) for either 1, 2, or 3 weeks [box plot: whiskers are defined at the minimum and maximum values, the top of the box is defined as the 75th percentile, the bottom of the box is the 25th percentile, and the middle line is defined as the median. * is defined as an extreme outlier. Different lowercase letters represent statistical differences between ammonia concentrations for each time period ($P < 0.05$, GEE, pairwise comparison with sequential Sidak correction, n = 6/ammonia concentration and time)].

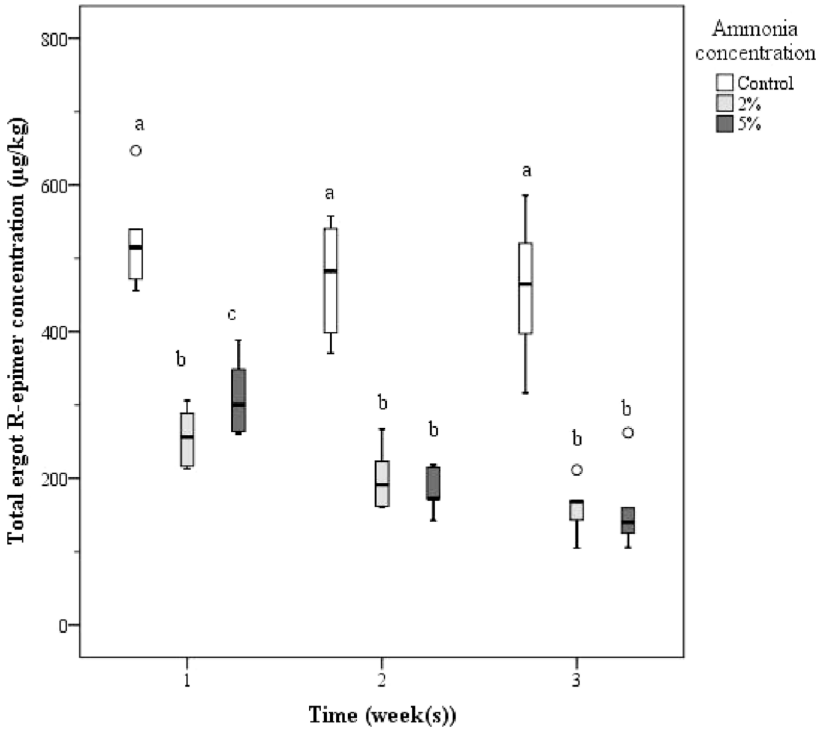

Figure 4. Total ergot *R*-epimer concentration at each time period and ammonia exposure. Total *R*-epimer concentration (μg/kg) in natural ergot-contaminated wheat after exposure to each concentration of ammonia (%) for either 1, 2, or 3 weeks [box plot: whiskers are defined as the minimum and maximum values; the top of the box is defined as the 75th percentile, the bottom of the box is the 25th percentile, and the middle line is defined as the median. ^{O} is defined as an outlier. Different lowercase letters represent statistical differences between ammonia concentrations at each time period ($P < 0.05$, GEE, pairwise comparison with sequential Sidak correction, n = 6/ammonia concentration and time)].

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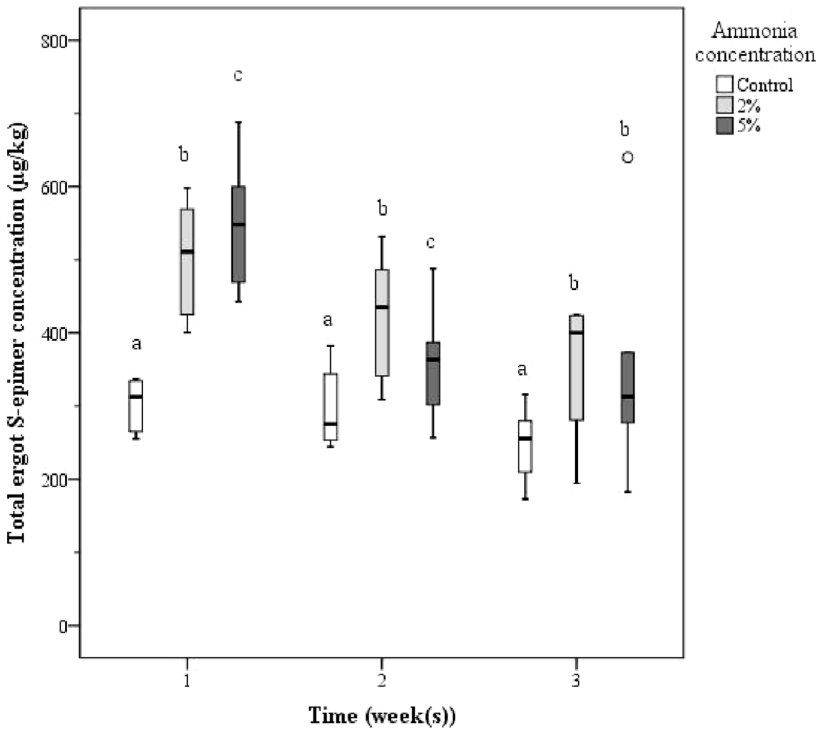

Figure 5. Total ergot *S*-epimer concentration at each time period and ammonia exposure. Total *S*-epimer concentration (μg/kg) in natural ergot-contaminated wheat after exposure to each concentration of ammonia (%) for either 1, 2, or 3 weeks [box plot: whiskers are defined at the minimum and maximum values, the top of the box is defined as the 75th percentile, the bottom of the box is the 25th percentile, and the middle line is defined as the median. ^{O} is defined as an outlier. Different lowercase letters represent statistical differences between ammonia concentrations at each time period ($P < 0.05$, GEE, pairwise comparison with sequential Sidak correction, $n = 6$/ammonia concentration and time)].

comparisons with sequential Sidak correction, $P \leq 0.002$). There was a range of 19−29% reduction in the mean total concentration of ergot epimers across all ammonia-exposed groups and time points (Figure 3).

The concentration of the total *R*- and total *S*-epimers, within the evaluated groups of control, 2% ammonia, and 5% ammonia in 1, 2, and 3 weeks, were normally distributed ($P > 0.05$). The *R*- and *S*-epimers responded differently when exposed to ammonia. There was a significant interaction between the effect of ammonia concentration and time on both mean total *R*-epimers and mean total *S*-epimers (GEE, Wald chi-square = 22.77 and 247.19 respectively, df = 4, $P < 0.001$ for both groups).

In weeks 1, 2, and 3, ammonia influenced the total *R*-epimer concentration (GEE, Wald chi-square = 451.76, 277.86, and 174.96, respectively, $P < 0.001$). Specifically, the total *R*-epimer concentration decreased from the control group at each ammonia concentration in each week (multiple pairwise comparisons with sequential Sidak correction, $P < 0.001$). There was a range of 40−66% reduction in the concentration of the mean total *R*-epimers (Figure 4).

In weeks 1, 2, and 3, ammonia influenced the total *S*-epimer concentration (GEE, Wald chi-square = 131.23, 29.90, and 31.79, respectively, $P < 0.001$). Specifically, the concentration of the total *S*-epimers increased in the 2 and 5% ammonia exposed groups (multiple pairwise comparisons with sequential Sidak correction, $P \leq 0.039$). There was a range of 21−81% increase in the mean total *S*-epimer concentration with ammonia concentration and time (Figure 5).

Ammonia appeared to affect the individual ergot alkaloids differently. As shown in Table 1, the response varied. At weeks 1, 2, and 3, for ergocornine/inine, ergocristine/inine, and ergocryptine/inine, the *R*-epimer concentrations decreased, and the *S*-epimer concentrations increased. The total concentration for ergocornine/inine, ergocristine/inine, and ergocryptine/inine decreased after exposure to 2% ammonia but increased after exposure to 5% ammonia compared to the control group in 1 week. In 2 and 3 weeks, the total concentration for those ergot alkaloids decreased after ammonia exposure, compared to the control group. The observed mean ratios for ergocornine/inine, ergocristine/inine, and ergocryptine/inine ratios all decreased below 1 for the 2 and 5% ammonia exposed groups, compared to control in all weeks. In week 1 for ergometrine/ergometrinine, ergotamine/ergotaminine and ergosine/ergosinine there did not appear to be a trend. However, in weeks 2 and 3, the concentration of both the *R*- and *S*-epimers of those ergot alkaloids in the ammonia-exposed groups decreased compared to the control group. Likewise, the total concentration of those ergot alkaloids also decreased. The observed mean *R*/*S* ratio for these ergot alkaloids decreased, however, not below 1, for all weeks analyzed. The ratios for all six samples from the ammonia groups exposed at each time, decreased, for all ergot alkaloids, except for one sample for ergometrine/ergometrinine in the 5% ammonia concentration group in week 2.

Ammonia−Epimer Degradation Products. Through comparing the chromatograms of the ammoniated ergocristine and ergocristinine standards to the respective control sample, a new product peak had formed. There was no difference in chromatograms between weeks 2 and 3. The control for ergocristine (*R*) had a product peak at 7.67 min with an m/z 610.39 with an abundance of 1.24×10^8 and a product peak at 7.90 min with an m/z 610.40 with an abundance of 3.08×10^6, corresponding to the *S*-epimer (Figure 6A). The ammoniated

https://doi.org/10.1021/acs.jafc.2c01583
J. Agric. Food Chem. 2022, 70, 8931−8941

Table 1. Concentration of the Six *R*- and Six *S*-Epimers of Ergot Alkaloids after Exposure to Control and 2 and 5% Ammonia for 1, 2, and 3 Weeks[a]

	week 1			week 2			week 3		
	control	2%	5%	control	2%	5%	control	2%	5%
ergocornine	42.34 ± 18.89	17.60 ± 6.71	21.24 ± 9.11	41.95 ± 18.36	12.60 ± 4.69	11.95 ± 5.02	38.34 ± 18.18	10.23 ± 4.70	10.72 ± 5.74
ergocorninine	35.46 ± 15.28	58.97 ± 23.46	67.09 ± 29.41	37.21 ± 17.03	47.87 ± 19.51	44.42 ± 22.17	29.82 ± 14.31	39.74 ± 21.26	42.43 ± 26.84
corTotal[b]	77.80 ± 34.17	76.57 ± 30.17	88.33 ± 38.52	79.15 ± 35.39	60.47 ± 24.20	56.37 ± 27.19	68.16 ± 32.48	49.98 ± 25.95	53.15 ± 32.58
corRS[c]	1.18 ± 0.12	0.30 ± 0.02	0.32 ± 0.02	1.14 ± 0.07	0.27 ± 0.04	0.29 ± 0.04	1.29 ± 0.08	0.28 ± 0.05	0.27 ± 0.04
ergocristine	266.51 ± 90.85	105.53 ± 24.92	135.09 ± 43.75	230.74 ± 64.08	89.20 ± 30.34	85.44 ± 24.02	231.06 ± 79.31	72.61 ± 28.73	72.12 ± 19.42
ergocristinine	161.13 ± 37.50	282.53 ± 73.52	314.66 ± 107.37	157.20 ± 33.12	260.57 ± 87.61	216.93 ± 63.33	131.21 ± 37.11	212.44 ± 71.91	208.70 ± 80.66
crisTotal[b]	427.64 ± 128.35	388.07 ± 98.45	449.75 ± 151.12	387.93 ± 97.20	349.77 ± 117.95	302.38 ± 87.34	362.27 ± 116.42	285.05 ± 100.64	280.82 ± 100.09
crisRS[c]	1.63 ± 0.19	0.38 ± 0.02	0.43 ± 0.04	1.46 ± 0.13	0.35 ± 0.04	0.40 ± 0.06	1.75 ± 0.13	0.35 ± 0.07	0.36 ± 0.08
ergocryptine	75.21 ± 18.14	32.31 ± 8.33	41.06 ± 12.22	70.71 ± 25.64	22.76 ± 5.11	22.52 ± 5.83	67.24 ± 24.10	17.75 ± 5.65	18.65 ± 8.04
ergocryptinine	45.40 ± 11.51	81.23 ± 18.69	90.82 ± 25.85	46.40 ± 20.26	64.15 ± 15.98	59.21 ± 18.11	37.79 ± 13.86	58.37 ± 22.60	58.25 ± 30.51
cryptTotal[b]	120.61 ± 29.65	113.54 ± 27.02	131.88 ± 38.07	117.11 ± 45.90	86.91 ± 21.09	81.74 ± 23.94	105.03 ± 37.96	76.12 ± 28.25	76.90 ± 38.55
cryptRS[c]	1.67 ± 0.20	0.40 ± 0.03	0.45 ± 0.03	1.56 ± 0.11	0.36 ± 0.03	0.39 ± 0.03	1.78 ± 0.14	0.31 ± 0.04	0.33 ± 0.05
ergometrine	35.56 ± 7.73	23.29 ± 1.65	25.64 ± 3.14	37.99 ± 9.54	14.85 ± 2.40	13.08 ± 3.88	30.51 ± 4.49	11.57 ± 2.74	10.37 ± 5.20
ergometrinine	13.67 ± 5.04	12.89 ± 0.83	14.68 ± 2.09	13.46 ± 2.49	8.86 ± 1.67	7.61 ± 1.62	10.04 ± 1.94	5.47 ± 1.38	5.04 ± 2.65
MetTotal[b]	49.23 ± 12.77	36.18 ± 2.48	40.32 ± 5.22	51.44 ± 12.02	23.72 ± 4.07	20.69 ± 5.50	40.55 ± 6.42	17.04 ± 4.12	15.41 ± 7.85
metRS[c]	2.74 ± 0.50	1.81 ± 0.07	1.75 ± 0.10	2.82 ± 0.46	1.71 ± 0.19	1.73 ± 0.35	3.08 ± 0.43	2.13 ± 0.12	2.07 ± 0.06
ergosine	23.52 ± 8.69	16.40 ± 3.74	20.64 ± 6.68	22.04 ± 10.18	13.93 ± 3.21	11.54 ± 2.74	20.64 ± 6.08	10.46 ± 3.32	10.14 ± 5.06
ergosinine	14.17 ± 5.03	14.13 ± 3.62	17.24 ± 6.67	14.58 ± 6.22	11.88 ± 3.14	9.17 ± 2.26	11.29 ± 3.31	8.52 ± 2.81	8.37 ± 4.43
sineTotal[b]	37.70 ± 13.72	30.53 ± 7.35	37.88 ± 13.35	36.62 ± 16.40	25.82 ± 6.35	20.71 ± 5.01	31.93 ± 9.39	18.98 ± 6.13	18.51 ± 9.49
sineRS[c]	1.65 ± 0.11	1.17 ± 0.17	1.24 ± 0.16	1.49 ± 0.11	1.18 ± 0.08	1.27 ± 0.09	1.84 ± 0.06	1.24 ± 0.07	1.24 ± 0.06
ergotamine	80.74 ± 19.36	60.99 ± 15.91	66.78 ± 20.58	68.67 ± 18.97	45.84 ± 17.90	37.34 ± 9.47	70.40 ± 22.50	38.17 ± 12.01	33.29 ± 18.39
ergotaminine	32.97 ± 7.16	52.57 ± 15.48	44.91 ± 13.25	27.14 ± 5.98	29.77 ± 10.54	22.98 ± 7.19	28.20 ± 10.22	29.50 ± 11.95	27.06 ± 22.30
TamTotal[b]	113.70 ± 26.52	113.55 ± 31.39	111.69 ± 33.83	95.81 ± 24.95	75.61 ± 28.44	60.32 ± 16.66	98.60 ± 32.72	67.66 ± 23.96	60.35 ± 40.68
tamRS[c]	2.45 ± 0.24	1.18 ± 0.14	1.49 ± 0.09	2.53 ± 0.38	1.56 ± 0.25	1.72 ± 0.33	2.60 ± 0.44	1.40 ± 0.31	1.44 ± 0.35
Total[d]	826.68 ± 94.43	758.44 ± 119.50	859.85 ± 139.08	768.07 ± 134.90	622.28 ± 129.87	542.2 ± 106.49	706.53 ± 142.07	514.83 ± 126.61	505.15 ± 211.28
TotalR[e]	523.88 ± 68.88	256.12 ± 40.22	310.45 ± 51.15	472.1 ± 82.58	199.18 ± 42.78	181.88 ± 29.42	458.20 ± 94.10	160.79 ± 35.25	155.29 ± 55.60
TotalS[f]	302.80 ± 35.12	502.32 ± 80.15	549.40 ± 89.88	295.97 ± 56.26	423.11 ± 89.36	360.32 ± 80.07	248.34 ± 51.62	354.04 ± 94.85	349.86 ± 156.25
TotalRSratio[g]	1.74 ± 0.18	0.51 ± 0.02	0.57 ± 0.03	1.60 ± 0.14	0.48 ± 0.04	0.51 ± 0.06	1.85 ± 0.15	0.47 ± 0.06	0.46 ± 0.06

[a]Values (μg/kg) are presented as mean ± standard deviation ($n = 6$). [b]The total concentration for each ergot alkaloid (*R*- + *S*-epimer concentration). [c]The ratio of the concentration for each individual *R*-/*S*-epimer pair for each ergot alkaloid. [d]Concentration of all *R*- and *S*-epimers from each of the six ergot alkaloids. [e]Concentration of all *R*-epimers from each of the six ergot alkaloids. [f]Concentration of all *S*-epimers from each of the six ergot alkaloids. [g]Ratio of the total *R*-epimer concentration to the total *S*-epimer concentration.

https://doi.org/10.1021/acs.jafc.2c01583
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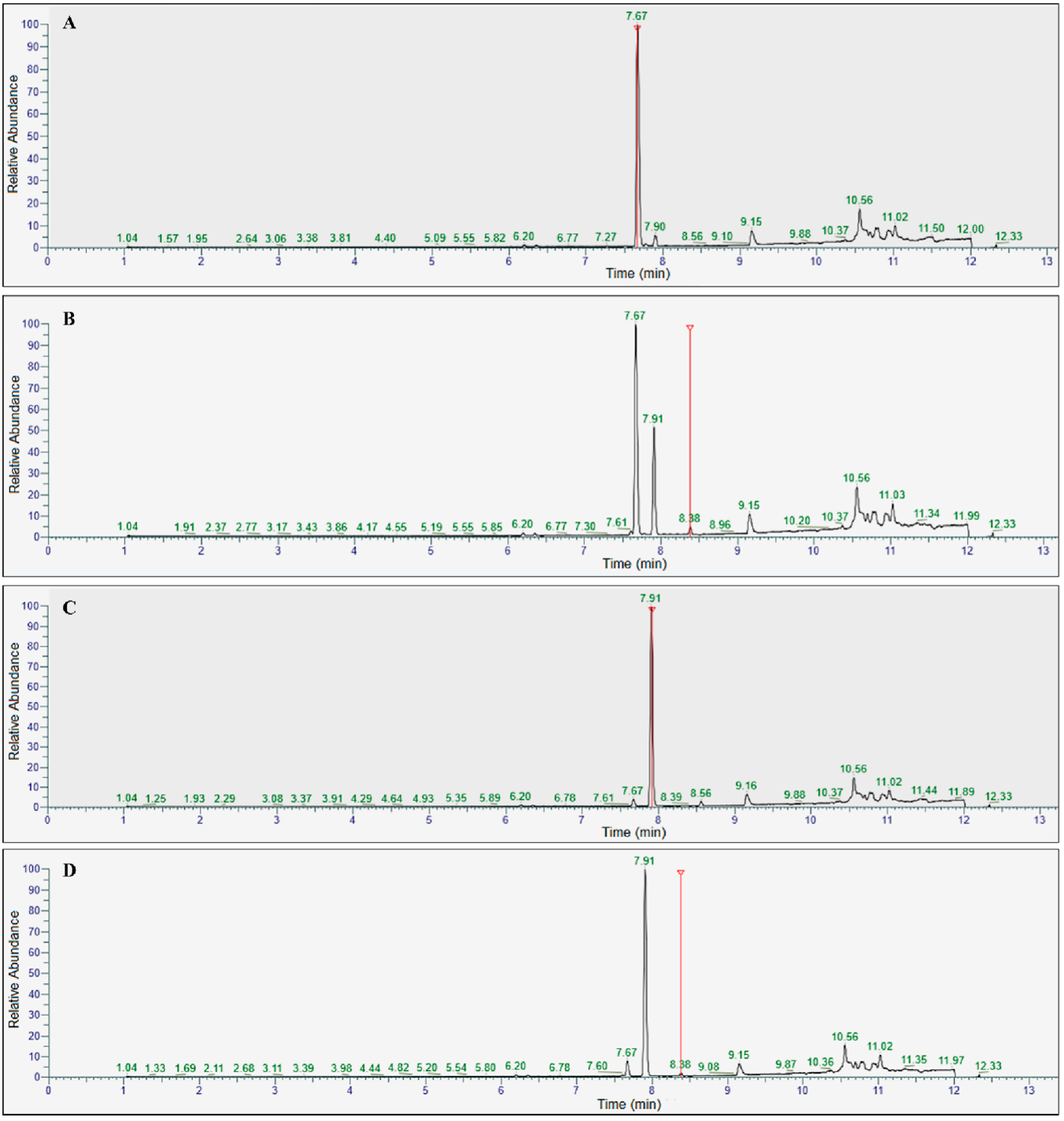

Figure 6. Extracted chromatograms of epimers exposed to ammonia. (A) Ergocristine (*R*) control, (B) ergocristine (*R*) ammonia-exposed, (C) ergocristinine (*S*) control, and (D) ergocristinine (*S*) ammonia-exposed.

ergocristine (*R*) still had a product peak at 7.67 min with *m*/*z* 610.39 with an abundance of 8.74×10^7 and a product peak at 7.91 min with *m*/*z* 610.38 with an abundance of 3.16×10^7. A new product peak was observed at 8.38 min with *m*/*z* 608.37 and 626.38 (Figure 6B). The control sample for ergocristinine (*S*) had a product peak at 7.91 min with *m*/*z* 610.39 with an abundance of 9.95×10^7 (Figure 6C). A product peak at 7.67 min was also observed with *m*/*z* 610.39 and an abundance of 4.66×10^6, corresponding to the *R*-epimer. The ammoniated ergocristinine (*S*) also had a product peak at 7.91 min with *m*/*z* 610.40 and an abundance of 9.42×10^7 and a product peak at 7.67 min with an *m*/*z* 610.40 with an abundance of 1.01×10^7. A new product peak at 8.38 min was observed with *m*/*z* 608.39 and 626.39 (Figure 6D).

The assessment of the *R*- and *S*-epimer standards exposed directly to NH_4OH resulted in complete degradation of the epimers. Peaks corresponding to ergocristine and ergocristinine (7.67 and 7.91 min with *m*/*z* 610) were not present in each of the samples. Major product peaks of the *R*- and *S*-epimer standards directly exposed to NH_4OH are 7.82 min with *m*/*z* 605.37 and 623.38, 7.90 min with *m*/*z* 624.37 and 642.37, 8.43 min with *m*/*z* 605.38 and 623.37, and 8.54 min

https://doi.org/10.1021/acs.jafc.2c01583
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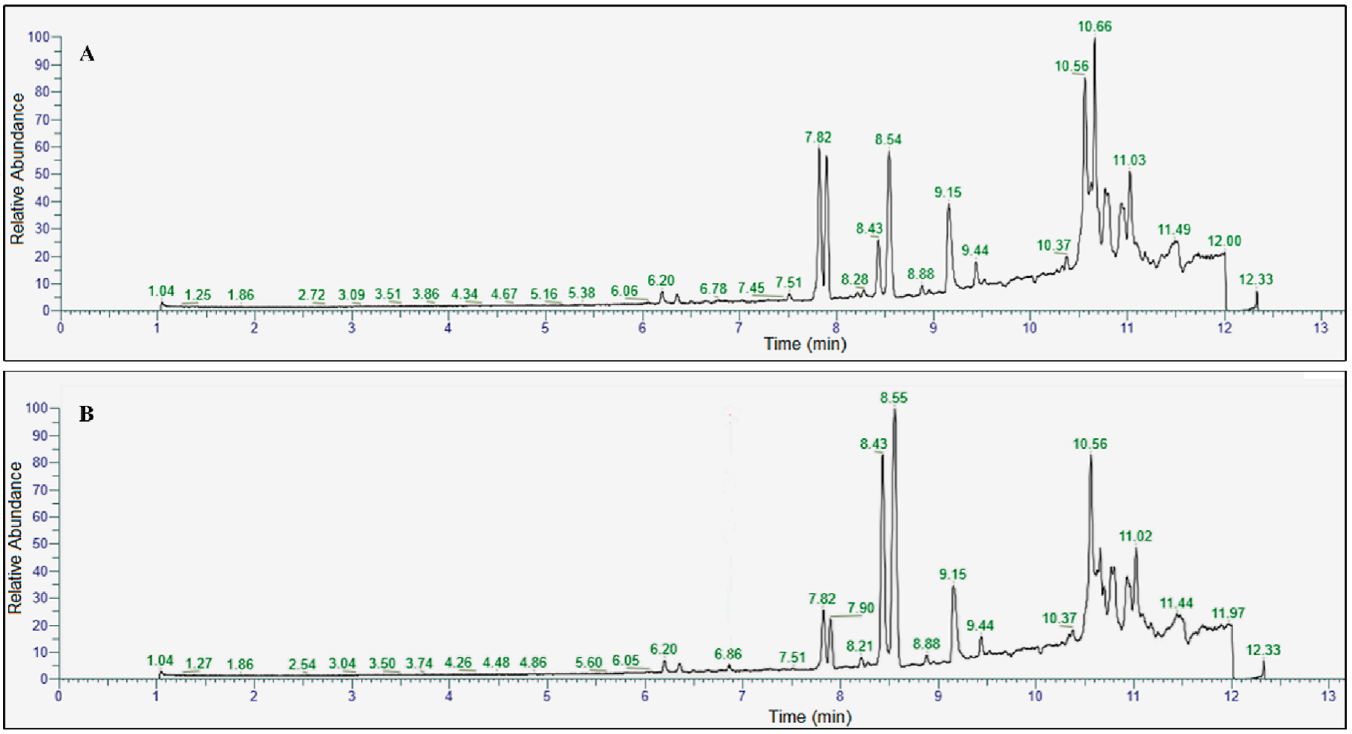

Figure 7. Extracted chromatogram of epimers exposed to NH_4OH. (A) Ergocristine (*R*) exposed to NH_4OH and (B) ergocristinine (*S*) exposed to NH_4OH.

with *m*/*z* 580.34. The ergocristine and the ergocristinine chromatogram exposed directly to NH_4OH do not appear to differ in product peaks; however, the relative abundance appears to be different (Figure 7A,B).

DISCUSSION

Ammonization has had optimistic effects on the reduction of multiple mycotoxins.[23] This study demonstrates that ammonia alters the total ergot epimer concentration. The total concentration of all ergot epimers decreased with exposure to both 2 and 5% ammonia at multiple time periods, resulting in potential detoxification. Similarly, a study also observed a decrease in the concentration of the total ergot alkaloids after ammonization compared to the control groups; however, it was not significant.[15] The authors utilized ELISA for their analysis, whereas the present study utilized a new method, and analysis was carried out using UHPLC−MS/MS. Potentially, the significant data could be a result of the use of a more sensitive and robust method/instrument and quantification of the *S*-epimers. Furthermore, another study used HPLC, and both ergovaline and ergovalinine were detected on the chromatogram, thereby utilizing total ergovaline.[14] The authors did not, however, quantify ergovalinine separately. They observed a decrease in the concentration of total ergovaline over weeks after exposure to ammonia, with no effect of ammonia concentration. In the present study, only in week 3, there was no difference in the total ergot epimer concentration between the 2 and 5% ammonia groups. Discrepancies between studies could be attributed to the instrument usage, analysis of one ergot alkaloid and not six as conducted in the present study, or the quantification of both the *R*- and *S*-epimers. A different study detected the *S*-epimers of ergot alkaloids using a HPLC system coupled to a fluorescence detector; however, it did not indicate the use of any *S*-epimer standards nor quantify the *S* configuration.[6] These authors did not observe differences in concentrations between ammoniated and nonammoniated groups. However, their short incubation period of 24 h may provide a rationale as to why there were no observed changes between groups. Comparatively, the present study analyzed the effects of ammonia over a longer time period. In the present study, it visually appears that the concentration of total ergot epimers decreases over time with ammonia treatment; however, temporal trends were not statistically assessed based on potential instrumental variation and natural changes in ergot concentration over time. The control groups were therefore analyzed at each time period to assess the effects of ammonia at each of the different times, and the changes were not due to instrumental or natural variability. Further experimentation would be needed to fully evaluate if ammonia exposure has a greater effect over time.

The total *R*- and total *S*-epimers, separately, in naturally contaminated grains, behaved differently after exposure to ammonia. The *R*-epimers of ergot alkaloids decreased in concentration, whereas the *S*-epimers increased in concentration, compared to the control group for both the 2 and 5% ammonia groups in all weeks analyzed. This observation suggests epimerization of the *R*-epimer to the *S*-epimer. The pH of the extraction solution for the analysis of ergot alkaloids is known to influence epimerization.[8] One study reported that the occurrence of the *S*-epimer was a result of the extraction procedure.[17] The present study had a pH extraction solution of approximately pH 6 for the control groups and pH 8 for the ammoniated groups; therefore, ammonia may influence the pH, which may seemingly encourage epimerization. Alkaline solutions have been said to encourage the epimerization from the *R*- to the *S*-epimer.[26] However, an extraction solvent of pH

https://doi.org/10.1021/acs.jafc.2c01583
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8.5 has been utilized to maximize extraction efficiency while minimizing epimerization.[25]

To further investigate if the pH influenced epimerization, the reconstituted solvents for the ammoniated and nonammoniated ergot epimer standards were tested using a Whatman Litmus paper for the pH status. The pHs of both ammoniated and nonammoniated samples were the same, while the results of the ammoniated ergot standards suggest epimerization of the *R*-epimer to the *S*-epimer, as indicated by comparing the chromatograms of the ammoniated and nonammoniated *R*-epimer standard. Therefore, ammonization may contribute to the epimerization of the *R*-epimer to the *S*-epimer and not only be attributed to the pH. However, this observation would have to be confirmed utilizing different methods. Regardless, the total *R*- and total *S*-epimers separately may react differently to ammonia, which may be influenced by specific ergot alkaloids.

Descriptive assessment of the concentrations of each ergot alkaloid separately indicates that specific ergot alkaloids may be more susceptible and react differently to ammonia. Ergocornine/ergocorninine, ergocristine/ergocristinine, and ergocryptine/ergocryptinine appear to have epimerized from the *R*-epimer to the *S*-epimer. This is associated with a decrease in the *R*-epimer concentration and an increase in the *S*-epimer concentration in 1, 2, and 3 weeks of exposure to ammonia. However, the total concentration of each of these ergot alkaloids decreased after exposure to ammonia at weeks 2 and 3. The *R*-epimers, of those ergot alkaloids, may be more susceptible to degradation/epimerization compared to the *S*-epimers. For ergometrine/ergometrinine, ergotamine/ergotaminine, and ergosine/ergosinine, in weeks 2 and 3, the concentrations of both the *R*- and *S*-epimers in the ammonia-exposed groups appear to have decreased compared to the control group. Likewise, the total concentration of these ergot alkaloids decreased. Potentially, these ergot alkaloids (*R*- and *S*-epimers) are less susceptible to epimerization after ammonia exposure than the ones described above. It appears that a contribution of epimerization and/or degradation occurred after exposure to ammonia for all ergot alkaloids analyzed. Analyzing all ergot alkaloids with epimer-specific quantification and not a single ergot alkaloid or configuration is supported.[14,15]

Ergot alkaloids may behave differently depending on their side group.[26] Structurally, this could be the rationale behind the observed differences between ergot alkaloids. Ergocornine/ergocornine, ergocristine/ergocristinine, and ergocryptine/ergocryptinine all have an isopropyl side group on the amino ring of the chemical structure.[18] Ergotamine/ergotaminine and ergosine/ergosinine both contain a methyl group on the amino ring structure of the molecule. Interestingly, the ergot alkaloids with similar side groups reacted similarly to ammonia exposure. A similar observation was seen after exposure to heat, ultraviolet light, and different pHs.[8] Therefore, the side groups of the ergot alkaloids may relate to their stability in terms of epimerization after exposure to various external factors.

Preliminary assessment of the nonammoniated and ammoniated ergocristine (*R*) and ergocristinine (*S*) standards suggest ammonia−epimer degradation products after ammonia exposure. The new product peaks of the ammoniated *R*- and *S*-epimer epimers eluted at the same retention time. The same retention time between ammoniated epimers suggests that only one epimer in each of the standards is ammoniated since the *R*- and *S*-epimers usually elute at different times. However, the ammonization process may change each of the initial epimers to the same configuration; therefore, both initial epimers may be ammoniated and elute at the same time. While this preliminary research suggests ammonia−epimer adducts, the occurrence of the ammonia−epimer reaction would need to be confirmed with other methods and on a larger scale. Based on the m/z values from the mass spectra of the ergot epimers exposed to ammonia vapors, or directly to ammonia hydroxide, it is speculated that ammonia would bind to the ergot epimers. This hypothesis is based on the chemical structure of the ergot epimers having multiple electrophilic centers available for a nucleophilic attack of an ammonia molecule. It is unknown where the ammonia molecules would bind and is out of the scope of this study. However, similar observations were noted with deoxynivalenol (DON)−ammonia degradation products,[23] in which the present study utilized similar methods. The authors of the DON−ammonia study created a hypothetical scheme of the addition of ammonia at a carbonyl group on the DON chemical structure, which represented the m/z value they observed in their mass spectra.[23] A similar reaction may have occurred in the present study. The authors also investigated the detoxification potential of the ammonia−DON degradation products using an *in silico* method.[23] The ammonia−DON compound was deemed as less toxic than the parent compound. Potentially, the suggested epimer−ammonia degradation products may also be less toxic, but further investigation is necessary.

Factors associated with the decreased ergot concentrations were based on practical applications, specifically, time and ammonia concentration. An incubation period of several weeks was utilized in the present study based on industry recommendations. In industry, low-quality forage is ammoniated for approximately 1−8 weeks depending on temperature.[27] Concentrations of 2 and 5% were utilized in the present study based on a practical application of 3% ammonia used for low-quality forage.[17] Ammonization can increase the nutritional value in low-quality feed. The process of ammoniating low-quality feed can be found online.[28]

If the low-quality feed is also contaminated with ergot, ammonization may cause detoxification. Previously, ammoniated ergot-contaminated feed resulted in mitigated toxic effects in cattle.[13] To further assess the detoxification potential of ammonization, quantification of both the *R*- and *S*-epimers of ergot alkaloids is necessary. Both configurations can cause adverse effects and are included in feed safety guidelines in several countries under the European Commission.[19,20,29] If ammonization of ergot-contaminated low-quality feed proves useful, the agriculture industry would have more available feed in years of ergot contamination and limited resources. Based on the present study, ammonization has the potential to be a practical detoxification method; however, optimal factors to facilitate ammonization are needed to assess true practicality on a larger scale.

In conclusion, the ammonization of natural ergot-contaminated wheat alters the concentration of the total, *R*-, and *S*-epimer concentrations. Exposure to ammonia caused a decrease in the total concentration of ergot epimers. However, the total concentration of *R*-epimers decreased, whereas the total concentration of *S*-epimers increased. Those observations appear to be influenced by the degradation/epimerization of specific ergot alkaloids and epimer configurations. Different ergot alkaloids (*R*- and *S*-epimer pairs) appear to behave

https://doi.org/10.1021/acs.jafc.2c01583
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differently after exposure to ammonia, potentially related to their structural differences. The use of UHPLC−MS/MS allows for an updated assessment of the impact of ammonia on ergot epimers and can quantify both configurations. Ammonization of *R*- and *S*-epimer standards suggests the addition of ammonia molecules to the chemical structure of an epimer. This may provide a rationale for the decrease in the total concentration of ergot epimers and supports the hypothesis that ammonization may contribute to epimerization. Ammonia, utilized in the agriculture industry, could potentially be useful for ergot detoxification, providing safer feed to livestock while increasing the nutritional value. Limitations of this study are the definitiveness of detoxification of ergot-contaminated grains for livestock consumption, toxicity/fate of hypothesized epimer−ammonia adducts, the nutritional value of the grain after ammonization, and low sample size, potentially resulting in missed noneffects and/or effects. The small scale of this study was utilized to investigate the potential use of ammonia to detoxify ergot in a lab setting, which needs to be researched further in a large-scale real-world setting. Another reason for the small scale of this study is also the ability to obtain independent ergot-contaminated samples of the same matrix. Future research should include a large-scale study to assess the practicality of ammoniating low-quality ergot-contaminated feed in a field setting while quantifying the *R*- and *S*-epimers of ergot alkaloids to meet feed guidelines. The effects of ammonia would need to be tested on the feed material with larger volumes to investigate if the ammonia reaction occurs. This research provides knowledge to further investigate practical ergot alkaloid detoxification mechanisms utilizing ammonia to ensure feed safety for animal consumption.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c01583.

Mass spectra of control and ammoniated ergocristine and ergocristinine (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC). They are grateful for the funding provided by the NSERC Canadian Graduate Scholarships-Doctoral (CGS-D), internal ergot grant from the Western College of Veterinary Medicine, and The Saskatchewan Ministry of Agriculture−Agriculture Development Fund grant number: 20180361. The authors would like to thank Prairie Diagnostic Services for the instrument usage and consumables, O. Collin for chromatogram assistance, the Canadian Feed Research Centre for the ergot-contaminated grains, Cherewyk Farms for the clean grains, and I. Moshynskyy for technical support and guidance.

■ ABBREVIATIONS

UHPLC−MS/MS, ultrahigh-performance liquid chromatography−tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; NH_4OH, ammonium hydroxide; NH_3, ammonia; QqQ, triple quadrupole; MS/MS, tandem mass spectrometer; GEE, generalized estimating equation; Q1, quadrupole one; DON, deoxynivalenol

■ REFERENCES

(1) Agriopoulou, S. Ergot Alkaloids Mycotoxins in Cereals and Cereal-Derived Food Products: Characteristics, Toxicity, Prevalence, and Control Strategies. *Agronomy* **2021**, *11*, 931.

(2) Cross, B. Experts fear ergot may become perennial problem. URL (https://www.producer.com/?s=experts+fear+ergot+may+become+perennial+problem+&c=n) (accessed on Aug 20, 2021).

(3) Canadian Grain Commission. *Official Grain Grading Guide*; Government of Canada, 2021. ISSN 1704-5118. https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/oggg-july-1-2021-en.pdf (accessed on Feb 22, 2022).

(4) Canadian Food Inspection Agency (CFIA). *RG-8 Regulatory Guidance: Contaminants in Feed* (formerly RG-1, Chapter 7), last revised October 16 2017. https://www.inspection.gc.ca/animal-health/livestock-feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=0 (accessed Aug 08, 2021).

(5) Koester, L. R.; Poole, D. H.; Serão, N. V. L.; Schmitz-Esser, S. Beef Cattle That Respond Differently to Fescue Toxicosis Have Distinct Gastrointestinal Tract Microbiota. *PLoS One* **2020**, *15*, No. e0229192.

(6) Young, J. C.; Chen, Z. J.; Marquardt, R. R. Reduction in Alkaloid Content of Ergot Sclerotia by Chemical and Physical Treatment. *J. Agric. Food Chem.* **1983**, *31*, 413−415.

(7) European Food Safety Authority (EFSA). Scientific Opinion on Ergot Alkaloids in Food and Feed. *EFSA J.* **2012**, *10*, 2798.

(8) Schummer, C.; Zandonella, I.; van Nieuwenhuyse, A.; Moris, G. Epimerization of Ergot Alkaloids in Feed. *Heliyon* **2020**, *6*, No. e04336.

(9) Merkel, S.; Dib, B.; Maul, R.; Köppen, R.; Koch, M.; Nehls, I. Degradation and Epimerization of Ergot Alkaloids after Baking and in Vitro Digestion. *Anal. Bioanal. Chem.* **2012**, *404*, 2489−2497.

(10) Bryła, M.; Ksieniewicz-Woźniak, E.; Waśkiewicz, A.; Podolska, G.; Szymczyk, K. Stability of Ergot Alkaloids during the Process of Baking Rye Bread. *Lebensm.-Wiss. Technol.* **2019**, *110*, 269.

(11) Tittlemier, S.; Drul, D.; Roscoe, M.; Turnock, D.; Taylor, D.; Fu, B. Fate of Ergot Alkaloids during Laboratory Scale Durum Processing and Pasta Production. *Toxins* **2019**, *11*, 195.

(12) Stanford, K.; Swift, M.; Wang, Y.; McAllister, T.; McKinnon, J.; Blakley, B.; Chaves, A. Effects of Feeding a Mycotoxin Binder on Nutrient Digestibility, Alkaloid Recovery in Feces, and Performance of Lambs Fed Diets Contaminated with Cereal Ergot. *Toxins* **2018**, *10*, 312.
(13) Chestnut, A. B.; Fribourg, H. A.; Gwinn, K. D.; Anderson, P. D.; Cochran, M. A. Effect of Ammoniation on Toxicity of Acremonium Coenophialum Infested Tall Fescue. *Anim. Feed Sci. Technol.* **1991**, *35*, 227−236.
(14) Simeone, A.-M. Ammoniation as a Detoxification Procedure for Endophyte-Infected Tall Fescue. Ph.D. Thesis, University of Kentucky: Lexington, KY, 1996. https://www-proquest-com.cyber.usask.ca/docview/304260997?pq-origsite=primo (accessed Aug 20, 2021).
(15) Roberts, C.; Kallenbach, R.; Hill, N. Harvest and Storage Method Affects Ergot Alkaloid Concentration in Tall Fescue. *Crop Manag.* **2002**, *1*, 1−3.
(16) Mandell, I. B.; Nicholson, H. H.; Christison, G. I.; NlG, C. Anim Sci, C. J. The Effects of Barley Processing on Nutrient Digestion within the Gastrointestinal Tract of Beef Cattle Fed Mixed Diets. *Can. J. Anim. Sci.* **1988**, *68*, 191−198.
(17) Spiegel, B. Ammoniated wheat straw boosts winter feed. *Success. Farming*, 2015; Vol. *30*. https://www.agriculture.com/livestock/cattle/feed/ammoniated-wheat-straw-boosts-winter_281-ar51805 (accessed Aug 08, 2021).
(18) Tittlemier, S. A.; Drul, D.; Roscoe, M.; Mckendry, T. Occurrence of Ergot and Ergot Alkaloids in Western Canadian Wheat and Other Cereals. *J. Agric. Food Chem.* **2015**, *63*, 6644−6650.
(19) Klotz, J. L.; Kirch, B. H.; Aiken, G. E.; Bush, L. P.; Strickland, J. R. Contractile Response of Fescue-Naïve Bovine Lateral Saphenous Veins to Increasing Concentrations of Tall Fescue Alkaloids. *J. Anim. Sci.* **2010**, *88*, 408−415.
(20) Cherewyk, J. E.; Parker, S. E.; Blakley, B. R.; Al-Dissi, A. N. Assessment of the Vasoactive Effects of the (S)-Epimers of Ergot Alkaloids in Vitro. *J. Anim. Sci.* **2020**, *98*, 1−6.
(21) Mulac, D.; Hüwel, S.; Galla, H. J.; Humpf, H. U. Permeability of Ergot Alkaloids across the Blood-Brain Barrier in Vitro and Influence on the Barrier Integrity. *Mol. Nutr. Food Res.* **2012**, *56*, 475−485.
(22) Cherewyk, J.; Grusie-Ogilvie, T.; Blakley, B.; Al-Dissi, A. Validation of a New Sensitive Method for the Detection and Quantification of R and S-Epimers of Ergot Alkaloids in Canadian Spring Wheat Utilizing Deuterated Lysergic Acid Diethylamide as an Internal Standard. *Toxins* **2022**, *14*, 22.
(23) Borràs-Vallverdú, B.; Ramos, A. J.; Marín, S.; Sanchis, V.; Rodríguez-Bencomo, J. J. Deoxynivalenol Degradation in Wheat Kernels by Exposition to Ammonia Vapours: A Tentative Strategy for Detoxification. *Food Control* **2020**, *118*, 107444.
(24) Lardy, G.; Bauer, M. Ammonisation of Low-Quality Roughages. URL (https://www.ndsu.edu/agriculture/ag-hub/ag-topics/livestock/nutrition-and-feeding/ammoniation-low-quality-roughages) (accessed Aug 08, 2021).
(25) Di Mavungu, J. D.; Malysheva, S. V.; Sanders, M.; Larionova, D.; Robbens, J.; Dubruel, P.; Van Peteghem, C.; De Saeger, S. Development and Validation of a New LC-MS/MS Method for the Simultaneous Determination of Six Major Ergot Alkaloids and Their Corresponding Epimers. Application to Some Food and Feed Commodities. *Food Chem.* **2012**, *135*, 292−303.
(26) Komarova, E. L.; Tolkachev, O. N. The Chemistry of Peptide Ergot Alkaloids. Part 1. Classification and Chemistry of Ergot Peptides. *Pharm. Chem. J.* **2001**, *35*, 504−513.
(27) Rasby, R. Ammoniating Crop Residues as a Feed Resource. URL (https://beef.unl.edu/cattleproduction/ammoniatingcropresidues#:~:text=The%20ammoniation%20process%20is%20temperature,for%202%20to%204%20weeks) (accessed Aug 08, 2021).
(28) Government of Saskatchewan. Ammonization of Straw and Chaff. URL (https://www.saskatchewan.ca/business/agriculture-natural-resources-and-industry/agribusiness-farmers-and-ranchers/livestock/cattle-poultry-and-other-livestock/cattle/feeding-livestock-during-feed-shortages/ammonization-of-straw-and-chaff) (accessed June 14, 2022).
(29) Commission Regulation (EU) 2021/1399 of 24 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs; Official Journal of the European Union, 2021. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32021R1399 (accessed March 01, 2022).

https://doi.org/10.1021/acs.jafc.2c01583
J. Agric. Food Chem. 2022, 70, 8931−8941