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ORIGINAL PAPER

Distribution of ergot alkaloids and ricinoleic acid in different milling fractions

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Abstract The sclerotia of the fungus *Claviceps* sp. are still a challenge for the milling industry. Ergot sclerotia are a constant contamination of the rye crop and have to be removed by modern milling technologies. Changing sizes and coloration of the sclerotia make it difficult to separate them from the grain. Ergot sclerotia are a problem when cleaning is insufficient and non-separated specimens or sclerotia fragments get into the milling stream and thus ergot alkaloids are distributed into the different cereal fractions. In model milling experiments, the residues of ergot in rye flour and the distribution of ergot into different milling fractions were investigated. Rye grains were mixed with whole ergot sclerotia and in another experiment with ergot powder and cleaned afterwards before milling. The ergot alkaloids ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristineand their related isomeric forms (-inine-forms), and additionally ricinoleic acid as a characteristic component of ergot, were quantified in the different milling fractions. From the first experiment, it can be shown that after harvesting even simple contact of sclerotia with bulk grains during ordinary handling or movement of bulk grain in the granary is sufficient to contaminate all the healthy or sound rye grains with ergot alkaloids. Thereby, the amount of ergot residue correlates with the amount of peripheral layers of rye grains in the flour. In an additional experiment without sclerotia specimens, bulk rye grains were loaded with powder of sclerotia. After subsequent cleaning, aconcentration of ergot alkaloids was detected, which was tenfold higher than the ergot alkaloidconcentration of the experiment with intact ergot sclerotia.

Keywords Ergot · Ergot alkaloids · Ricinoleic acid · Rye · Grain milling

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Introduction

In Germany and some eastern and northern European countries, rye is the most important bread-making raw material after wheat. Especially in Germany, a broad variety of rye products is available. The per capita consumption in 2008/2009 was 8.9 kg (Verband Deutscher Mühlen 2009). The crop yields of rye can be increased by planting hybrid rye varieties. A disadvantage of these hybrids is that, due to the smaller supply of pollen, the risk of infection with ergot is higher (Mielke 2000; Wortmann 2005). Consequently, ergot is still a permanent contamination problem of the rye crop. Although the mills with their modern milling technologies are able to remove the ergot sclerotia from the bulk grains, there still remain residues of ergot and thus ergot alkaloids are detectable (Münzing et al. 2004). Ergot alkaloids are secondary metabolites produced by the fungus *Claviceps* sp. The most prominent alkaloids in occurrence and toxicity are the lysergic acid alkaloids ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine and their related isolysergic acid forms, the 8-(S)-epimers or -inine forms. The chemical structures are shown

in Fig. 1. These ergot alkaloids are responsible for the diseases related to ergot-contaminated grain, called ergotism, which occurred in particular in the Middle Ages. The amount of alkaloids in the sclerotia varies considerably. An average amount of 0.2% (0.01–0.5%) is given by Lorenz (1979). However, our own measurements of the ergot alkaloid contents of 63 ergot sclerotia samples resulted in an average amount of 0.08% (757 mg/kg) (Franzmann et al. 2010).

At present, there is no legal regulation in the European Union (EU) for the alkaloid content but there is for the amount of ergot impurities in feed materials containing unground cereals (Directive 2002/32/EC) (The European Parliament and the Council of the European Union 2002). The maximum level is set to 0.1% ergot sclerotia present in feed material. For food, there is actually no limit, either for the alkaloid content or for the amount of ergot impurities. A reference value of 0.05% as the maximum amount of ergot sclerotia impurities in rye products exists according to both good agricultural practice and good manufacturing practice. An objective of this study was to follow the ergot content and contamination from the raw grain through the cleaning processes into the milling fractions. Therefore, a model grain cleaning and milling experiment was performed. Additionally, samples from a commercial rye mill were analyzed.

The amount of ergot in rye products was determined on the basis of the ergot alkaloids and the ricinoleic acid [(R)-12-hydroxy-(Z)-9-octadecenoic acid] as it is a characteristic fatty acid of ergot oil. The ricinoleic acid amount in the fat is given as approximately 30% (Bharucha and Gunstone 1957; Mantle et al. 1969; Morris and Hall 1966; Whittemore et al. 1976).

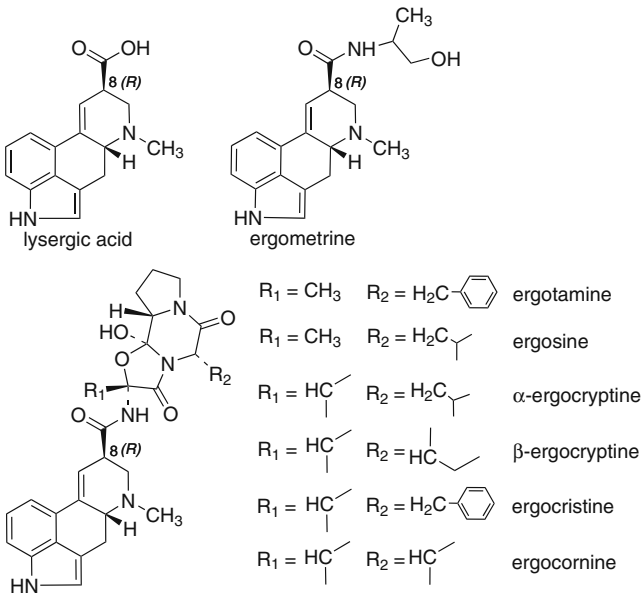

Fig. 1 Chemical structures of lysergic acid ergot alkaloids

Our recent investigations have shown that the ricinoleic acid concentration (average amount in the ergot sclerotia: 103 g/kg, n=55) correlates with the total ergot content (Franzmann et al. 2010).

Materials and methods

Materials

Ergot sclerotia from rye and whole rye grain samples were kindly provided by the Carl Mühle, Untersiemau, Germany.

Chemicals and reagents

15-Hydroxypentadecanoic acid was purchased from Alfa Aesar (Karlsruhe, Germany). Ricinoleic acid, ergometrine maleate (=ergonovine maleate), ergotamine-D-tartrate, α-ergocryptine and ergocornine were purchased from Sigma-Aldrich (Steinheim, Germany). Ergosine, ergosinine, ergocristine, ergocristinine, ergometrinine, ergotaminine, ergocorninine, α-ergocryptinine were obtained from Alfarma (Černošice, Czech Republic). Methysergide maleate was from Biotrend (Wangen, Switzerland). α-Amylase was purchased as powder (35 U/mg) from Fluka (Sigma-Aldrich) and dissolved in water or as solution (Termamyl 120 KNU/G) from Novo Nordisk (Bagsvaerd, Denmark). Ammonium carbamate, N,O-bis (trimethylsilyl)acetamide, trimethylchlorosilane, trimethylsilylimidazole were from Fluka (Sigma-Aldrich). Sodium hydroxide pellets, hydrochloric acid and aqueous ammonia solution (25%) were from Grössing (Filsum, Germany). 1,1,2-Trichloro-1,2,2-trifluoroethane was purchased from LGC Promochem (Wesel, Germany). Orthophosphoric acid, ethyl acetate, 1-butanol, toluol and tert-butylmethylether were from Roth (Karlsruhe, Germany).

Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Purified water was generated by a Milli-Q Gradient A10 system (Millipore, Schwalbach, Germany). Solid phase extraction cartridges Bond Elut AL-B and Bond Elut NH2 (sorbent weight 500 mg) were purchased from Varian (Darmstadt, Germany). Syringe filters type "Rotilabo" with PVDF membrane with 0.45 µm pore size and 15 mm diameter were purchased from Roth.

Methods

Model milling experiments To contaminate rye kernels artificially, 15 kg of rye grain and 15 kg of sorting out liftings with an ergot sclerotia amount of 24% were mixed for 1/2 h in a tumbling mixer. Afterwards, this mixture was cleaned by use of: first, a Dockage Tester (Brabender, Duisburg, Germany), which is a laboratory grain cleaning

machine with special oscillating riddles and triangular sieves to separate impurities from the grain; second, a Labofix 90 (Brabender), combining all mechanical processes including air separators, slotted screen sieves, and indented cylinders; and third, an ESM3 Vision (ESM/Satake, Bredbury, UK), which separates by optoelectronic detection. For the last cleaning step, residual impurities of intact and broken sclerotia were separated manually.

For the first experiment (experiment 1, see Fig. 2), this cleaned rye was moistened to a water content of 15.5% for 2 h and afterwards crushed and then ground by corrugated rolls according to the German milling standard for rye with a Bühler laboratory scale mill (MLU-202; Bühler, Braunschweig, Germany). Meal flour fractions B1, B2 and B3 and the reduction flour fractions C1, C2 and C3 were obtained. As prescribed in the standard methods, the bran was finished by an impact mill (MLU-302; Bühler) to get the adhering flour from the bran (SM1 and SM2) (Arbeitsgemeinschaft Getreideforschung e.V 1994). The milling scheme of the roller mill is given in Fig. 3.

For the second experiment (experiment 2a), 3 kg of the completely cleaned rye were mixed with 30 g ergot powder (ergot ground in an impact mill to a particle size <500 μm) for 3 min in a tumbling mixer. This mixture was then cleaned using the Labofix, moistened to a water content of 15.5% for 2 h and afterwards crushed and then ground into the meal flour fractions B1, B2 and B3 and the reduction flour fractions C1, C2 and C3. The bran was finished twice in a bran finisher, resulting in bran and two flours (SM1 and SM2). For the next experiment (experiment 2b), 4 kg of the cleaned rye were mixed with 40 g ergot powder (ergot sclerotia ground in an impact mill to a particle size <500 μm) for 3 min in a tumbling mixer. After the cleaning step using the Labofix, the rye was moistened for 10 min and then peeled in a grain peeler (DSRH; Bühler) whereby the pericarp with the adhering ergot dust was erased. Before squeezing, the water content was set to 15.5%. The same milling fractions as mentioned above were obtained. A scheme of this procedure is given in Fig. 2. The ergot alkaloid contents and the ricinoleic acid contents of the milling fractions, of the crushed rye, and of the ergot sclerotia were determined.

Analysis of ricinoleic acid Ricinoleic acid was analyzed with GC-FID and quantified by use of the internal standard 15-hydroxypentadecanoic acid after hydrolysis of the fat and extraction of the fatty acids (Franzmann et al. 2010).

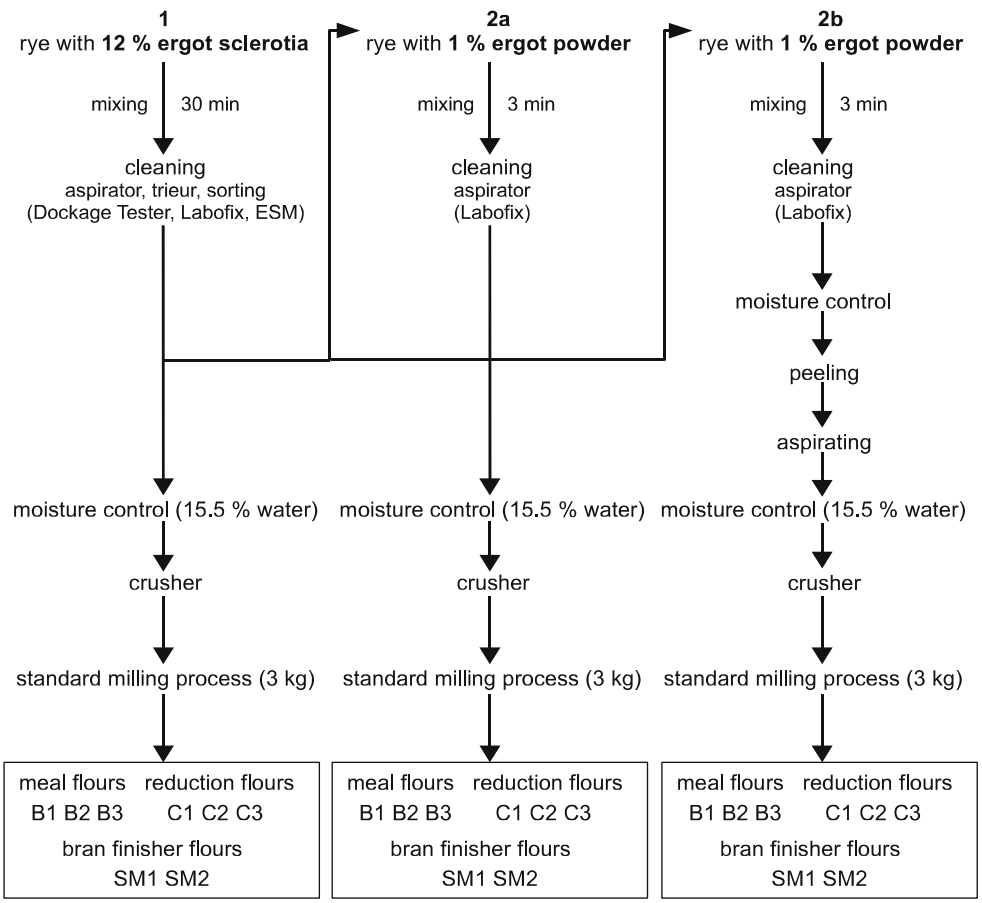

Fig. 2 Scheme of the experimental procedures

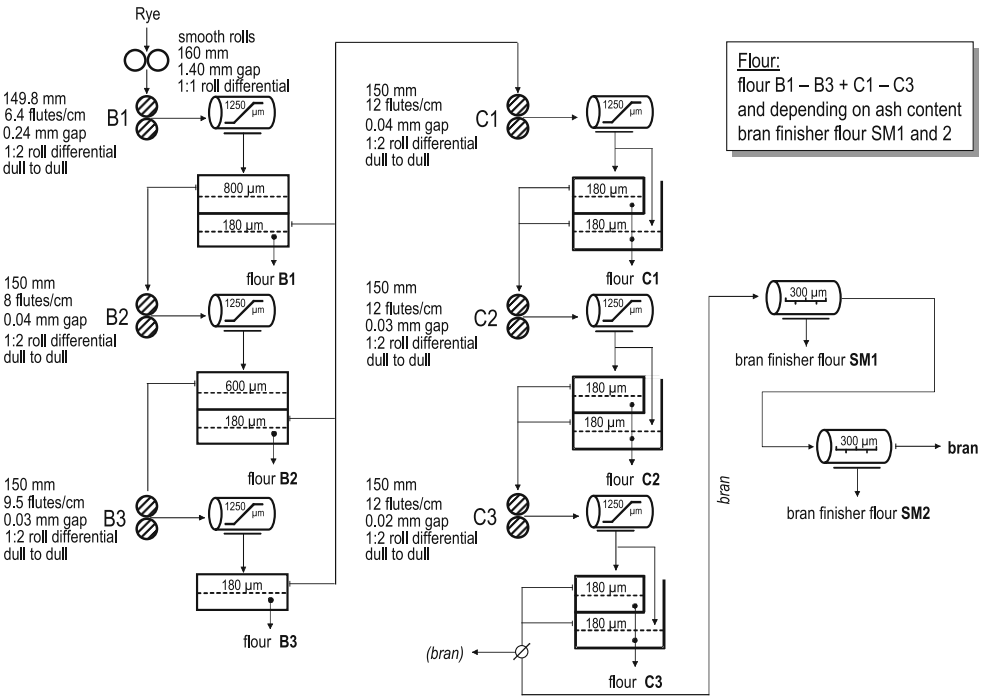

Fig. 3 Milling scheme of the laboratory scale roller mill for rye, *B1–B3* meal flours, *C1–C3* reduction flours, SM1+2 bran finisher flours

Analysis of ergot alkaloids Ergot alkaloids were analyzed by use of HPLC-FLD and quantified by means of the internal standard methysergide after an alkaline organic extraction according to Möller et al. (2009). The total ergot alkaloid contents were calculated by summing the amounts of the alkaloids ergometrine, ergosine, ergotamine, ergocornine, α-ergocryptine, ergocristine and their related -inine forms.

Sample preparation was twofold and the subsequent measurement was onefold each. The standard deviations (SD) result from the replicated samples.

Analytics

GC-FID A HP 6890 series gas chromatograph with flame ionization detector (Hewlett-Packard/Agilent, Böblingen, Germany) was used. The chromatographic separation was performed on a 30 m×0.25 mm i.d. fused silica, 0.1 µm Rtx-35 column (Restek, Bad Soden, Germany), using 0.6 mL/min hydrogen as carrier gas. The injector temperature was set to 280°C, injection volume was 1 µL with split injection (1:10 for ergot samples, 1:5 for rye samples). The column temperature was held at 200°C for 2 min in the beginning and then increased by 10°C/min to 240°C for 2 min and by 60°C/min to 300°C for 5 min. The detector temperature was set to 300°C. Data acquisition was performed with the Chemstation software (Agilent). Quantitation was carried out by peak area and internal standard calibration with 15-hydroxypentadecanoic acid as internal standard.

HPLC-FLD The sample extracts were dissolved in acetonitrile/ammoniumcarbamate buffer (50:50, v/v) and the ergot alkaloids separated on a 250×4.6 mm i.d., 5 µm, Omnispher C18 column (Varian). The HPLC-system consisted of a binary pump (L-7100; Merck-Hitachi, Tokyo, Japan), an autosampler (AS-2000A; Merck-Hitachi) and a fluorescence detector (FLD F-1050; Merck-Hitachi). The injection volume was 10 µL. The binary mobile phase consisted of acetonitrile (mobile phase A) and ammonium carbamate buffer, 0.2 g/L (mobile phase B). The initial gradient conditions were 35% mobile phase A which was increased at a linear rate to 60% over the next 18 min, held for 1 min and then increased further to 70% over 3 min, held for 4 min and finally equilibrated to the starting conditions for 8 min with a total run time of 35 min. The flow rate was 1 mL/min. The ergot alkaloids were detected with a fluorescence detector at an excitation wavelength of 330 nm and an emission wavelength of 415 nm. Data acquisition was performed with Merck-Hitachi D-7000 HSM HPLC System Manager software. Quantitation was carried out by peak area and internal standard calibration with methysergide as internal standard.

Results and discussion

Due to the fact that many different charges of breadcereal rye are blended in the mill, we decided to perform a model milling experiment with defined bulk good of rye and ergot sclerotia.

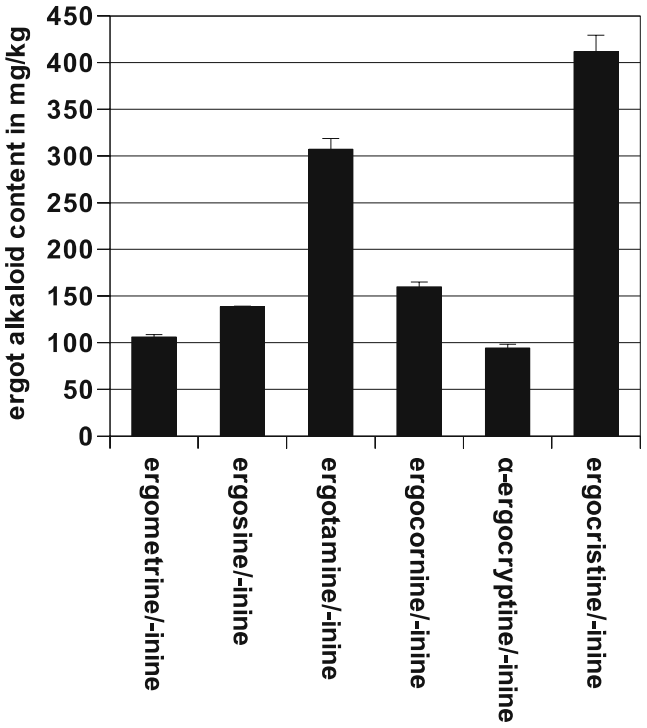

Fig. 4 Single ergot alkaloid contents of the used ergot sclerotia; total ergot alkaloid content 1,218 mg/kg

The total ergot alkaloid content of the ergot sclerotia used for the mixture was 1,218 mg/kg and the ricinoleic acid content was 106 g/kg. The main alkaloids were ergotamine and ergocristine. The percentage of ergotamine of the total ergot alkaloid content was 20.1% and of ergocristine 26.2%. The sum of ergotamine, ergtoaminine, ergocristine and ergocristinine was 59.0% of the total alkaloid content. The amounts of each single ergot alkaloid are shown in Fig. 4. In the rye itself, no ergot alkaloids were detectable regarding to a limit of quantification of 1.1–6.7 µg/kg for each single ergot alkaloid (Franzmann et al. 2010).

Table 1 Yield of the milling fractions in relation to the ergot alkaloid content: experiment 1 rye mixed with 12% ergot sclerotia, cleaned, ground

Milling fraction	Yield of flour in g	Yield of flour in %	Total ergot alkaloid content in µg/kg	Total ergot alkaloid content corrected by yield in µg/kg
B1	550	19.5	139	27.1
B2	346	12.2	155	18.9
B3	122	4.3	229	9.9
C1	506	17.9	289	51.7
C2	222	7.9	472	37.3
C3	113	4.0	613	24.6
SM1	375	13.3	1,002	133.3
SM2	145	5.1	1,462	74.6
Bran	447	15.8	239	37.8
Rye after crusher			356	
Sum	2,826	100		415

Table 2 Yield of the milling fractions in relation to the ergot alkaloid content: experiment 2a rye mixed with 1% ergot powder, cleaned, ground

Milling fraction	Yield of flour in g	Yield of flour in %	Total ergot alkaloid content in µg/kg	Total ergot alkaloid content corrected by yield in µg/kg
B1	544	19.4	5,437	1,055
B2	339	12.1	3,064	371
B3	147	5.3	1,822	97
C1	507	18.1	3,993	723
C2	207	7.4	3,499	259
C3	107	3.8	3,567	136
SM1	366	13.1	4,723	619
SM2	143	5.1	7,313	373
Bran	440	15.7	4,050	636
Rye after crusher			5,084	
Sum	2,800	100		4,267

The model milling experiments show that, even after careful cleaning with common milling technologies, residues of ergot still adhere to the rye grains. Whereas whole sclerotia could be removed effectively, ergot dust remains entangled in the inward surface crease and in the ”brush” of the rye kernels and is therefore very difficult to remove.

During the cleaning procedure of experiment 1 (Fig. 2), the ergot amount in the rye–sclerotia mixture could be reduced from 12% in the raw material to 0.03% (total alkaloid content 356 µg/kg, SD 16 µg/kg) in the crushed rye. In the batch of rye which was mixed with 1% of ergot powder an amount of 0.4% ergot (total alkaloid content

Table 3 Yield of the milling fractions in relation to the ergot alkaloid content: experiment 2b rye mixed with 1% ergot sclerotia, cleaned, peeled, ground

Milling fraction	Yield of flour in g	Yield of flour in %	Total ergot alkaloid content in µg/kg	Total ergot alkaloid content corrected by yield in µg/kg
B1	602	21.7	2,265	492
B2	349	12.6	1,467	185
B3	160	5.8	9,56	55
C1	456	16.5	1,272	210
C2	197	7.1	1,468	104
C3	97	3.5	1,361	48
SM1	346	12.5	2,004	251
SM2	139	5.0	2,406	120
Bran	422	15.2	1,437	218
Rye after crusher		1,614		
Sum	2,768	100		1,683

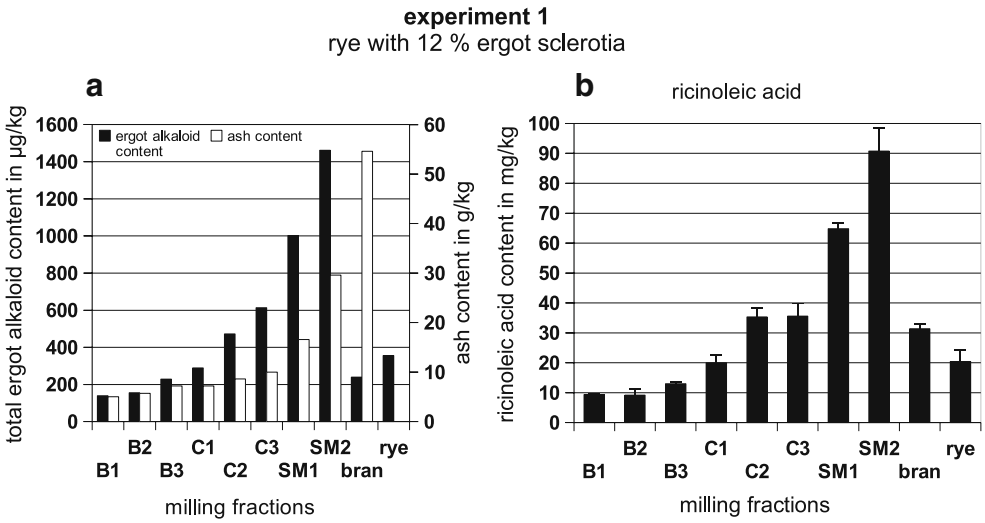

Fig. 5 Ergot alkaloid and ricinoleic acid contents in the milling fractions: experiment 1, rye mixed with 12% ergot sclerotia, cleaned, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

5,084 µg/kg, SD 82 µg/kg) remained after the described cleaning procedure (experiment 2a, Fig. 2). Due to the additional peeling step in experiment 2b (Fig. 2), the ergot amount in the crushed rye was reduced from 1% to 0.1% (total alkaloid content 1,614 µg/kg, SD 75 µg/kg).

There were no differences in the yields of flour between the experiments. The yield of flour was 84% without the bran in each experiment. The yields of the different milling fractions are given in Table 1 (experiment 1), Table 2 (experiment 2a) and Table 3 (experiment 2b). An interesting point was to investigate the distribution of ergot, i.e. ergot alkaloids and ricinoleic acid, in the different milling fractions. Depending on the designated flour type, differing amounts of each milling fraction are merged. For example, for rye flour type 997 with an ash content of 0.997% (DM), 80–85% of the inner rye grain are used.

The rye grinding with corrugated rolls followed the milling scheme given in Fig. 3. This continuous milling diagram implicates an increasing degree of fineness from the milling fraction B1 (first meal flour) to C3 (last reduction flour) which correlates with an increasing amount of peripheral compounds of the kernel in the flour. As can be seen from Fig. 5, the amounts of ergot alkaloids and ricinoleic acid likewise increase with the higher amount of the bran layers in the flour. The total ergot alkaloid content in the first meal flour (B1) was 139 µg/kg (SD 0.9 µg/kg) and increased to 613 µg/kg (SD 2.7 µg/kg) in the last reduction flour (C3) and 1462 µg/kg (SD: 30.3 µg/kg) in the second flour of the bran finisher (Table 1). Additionally the ash contents and alkaloid contents of the milling fractions of experiment 1 are shown exemplarily in Fig. 5a. Hence one can conclude that the contamination with ergot alkaloids is ascribed to the abrasion of the ergot sclerotia to the peripheral layers of rye grain. Scott and Lawrence (1980) and Baumann et al. (1985) analyzed various wheat and rye flour samples and also observed higher ergot alkaloid amounts with an increasing degree of milling. Total ergot alkaloid amounts in rye flours ranged from 14.5 to 397.4 µg/kg.

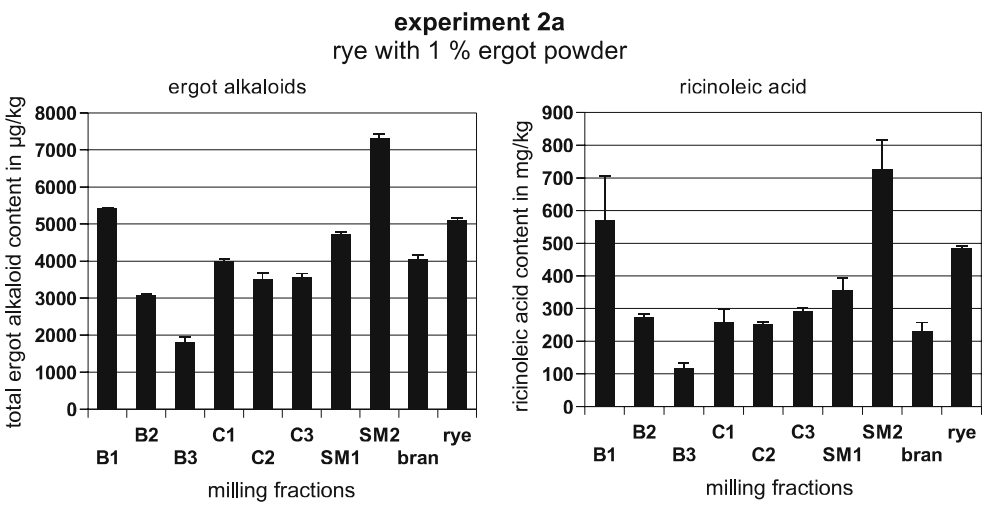

Fig. 6 Ergot alkaloid and ricinoleic acid contents in the milling fractions: experiment 2a, rye mixed with 1% ergot sclerotia, cleaned, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

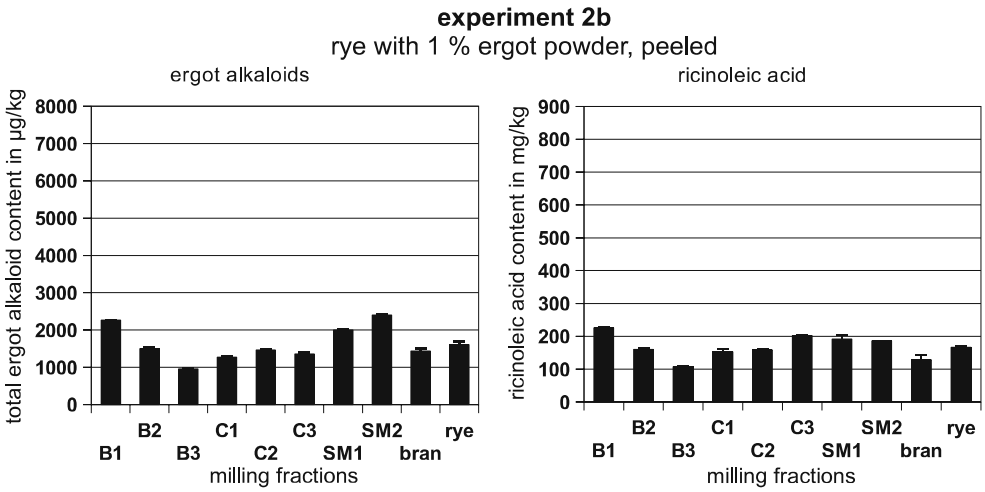

Fig. 7 Ergot alkaloid and ricinoleic acid contents in the milling fractions: experiment 2b, rye mixed with 1% ergot sclerotia, cleaned, peeled, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

The results of the second experiment (experiment 2a) are given in Fig. 6. The first obvious difference to experiment 1 is that the total ergot alkaloid and ricinoleic acid amounts were much higher. This demonstrates that the ergot dust is the main problem in grain cleaning processes. Moreover, the distribution was completely different. With regard to the meal and semolina flours, the highest amount of ergot was located in the first meal flour (B1, total ergot alkaloid content 5,437 µg/kg, SD 55.6 µg/kg) and the lowest amount was in the meal flour from the third break (B3, total ergot alkaloid content 1,822 µg/kg, SD 124 µg/kg). Between the reduction flours were only slight differences (C1–C3, total ergot alkaloid contents 3,993 µg/kg, SD 57 µg/kg, 3,499 µg/kg, SD 174 µg/kg, 3,567 µg/kg, SD 89 µg/kg). The flour from the bran finisher after the second peel off (SM2) contained the highest ergot concentration in all experiments. Thus, the ergot particles were separated from the bran by bran finishing. By the additional peeling step in experiment 2b the amount of ergot was reduced to one third. The distribution of ergot in the milling fractions was quite similar to experiment 2a (Fig. 7). Bran finishing means a dynamic abrasive impact on the bran and the adhering endosperm particles (SM1 and SM2). In addition to this effect, the abrasive forces also peel off the adhesive ergot contamination from the bran. This results in lower total ergot alkaloid levels in the bran compared to the value of intact rye kernels (Figs. 5, 6 and 7). The approach to explain the very high total ergot alkaloid content of the B1 flour is that the impurities located in the crease of the kernel get into the flour of the first break (B1) as usual.

Regarding the amounts of the single ergot alkaloids in each milling fraction there were some remarkable differences between the experiments. Whereas in experiments 2a and 2b, with the ergot added as powder, the single alkaloids were distributed almost equally between the milling fractions, the alkaloid distribution of experiment 1 was more divergent. The strongest differences were observed in the amounts of the crushed rye and the bran (Figs. 8, 9 and 10).

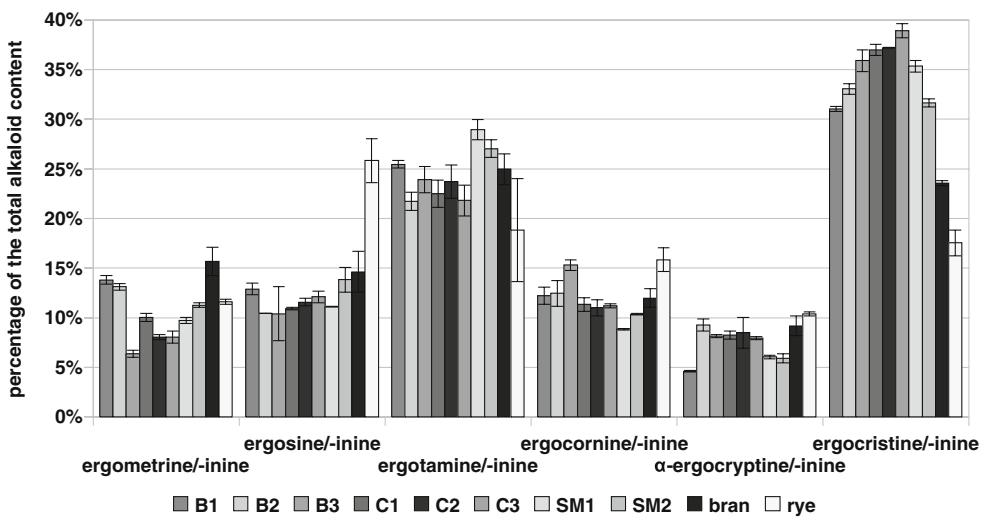

Fig. 8 Distribution of the single ergot alkaloid contents in the milling fractions (percentage of the total alkaloid content): experiment 1, rye mixed with 12% ergot sclerotia, cleaned, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

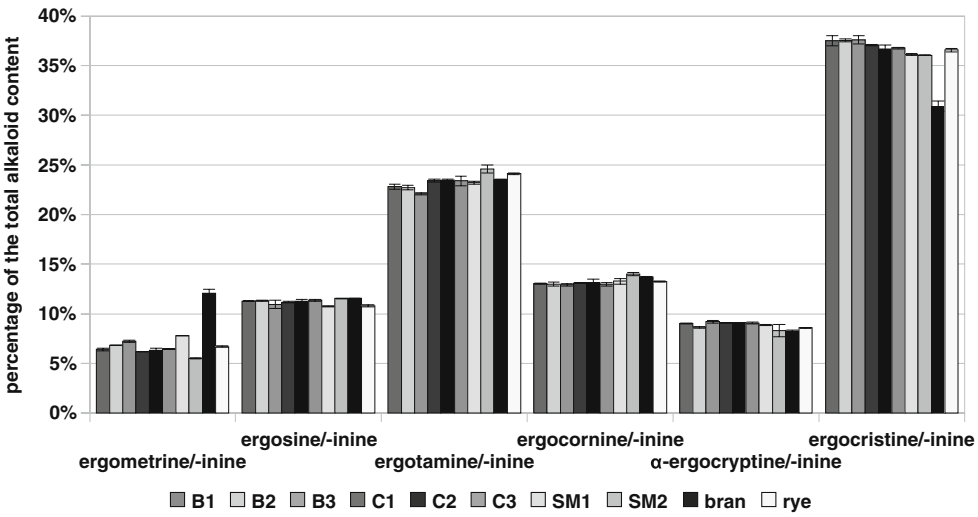

Fig. 9 Distribution of the single ergot alkaloid contents in the milling fractions (percentage of the total alkaloid content): experiment 2a, rye mixed with 1% ergot powder, cleaned, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

The equal distribution of the alkaloids in the milling fractions of experiments 2a and 2b indicates that there was less interaction between the added powder and the rye grains. Experiment 1 examines the abrasive effect of components of the ergot sclerotia on the rye grain surfaces in a realistic way. The main alkaloids ergotamine and ergocristine, for example, were mainly found in the bran finisher flours and the lowest amount was in the crushed rye. Hardly any differences were detectable in experiments 2a and 2b.

Wolff et al. (1983) investigated the distribution of ergometrine in different milling fractions. They added various amounts of ergot to a collective of kernels before grinding. Ergometrine was chosen as indicator for the ergot amount in the milling fractions. They found an amount of 20% ergot in the bran and more than 70% in the flours. As our results from experiment 1 show, the distribution of each alkaloid in the milling fractions is slightly different. Ergometrine on the one hand accumulated in the bran, ergocristine on the other hand was degraded (Fig. 8).

As the yields of the single milling fractions are not equal (meal flour B1 and meal flour C1 are the main milling products), the yields of flour and the alkaloid contents are summarized in Table 1 (experiment 1), Table 2 (experiment 2a) and Table 3 (experiment 2b). The sum of the calculated alkaloid contents (column 5) almost corresponds to the measured alkaloid content of the crushed rye (column 4).

The results of the analyzed samples from a rye mill underline the hypothesis that the ergot alkaloid concentration rises with increasing degree of fineness, i.e. the amount of peripheral layers in the flour. In the sample of the first meal flour of this mill, ergometrine and ergocristine were detectable but the concentrations were below the limit of quantification. The total ergot alkaloid content of the last reduction flour was 166 µg/kg (SD 42 µg/kg) (data not shown).

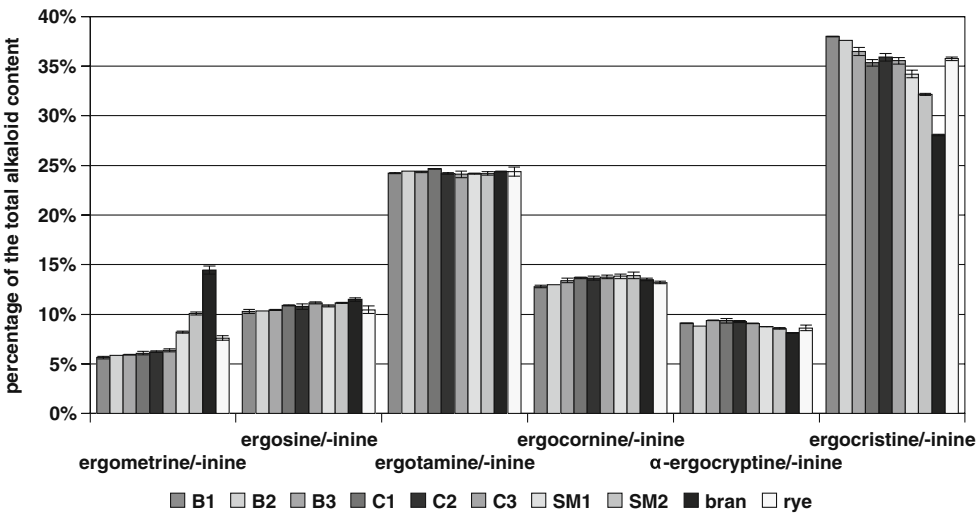

Fig. 10 Distribution of the single ergot alkaloid contents in the milling fractions (percentage of the total alkaloid content): experiment 2b, rye mixed with 1% ergot powder, cleaned, peeled, milled to flours. *B1–B3* meal flours, *C1–C3* reduction flours, *SM1–SM2* flours of the bran finisher

Conclusion

With the model milling experiments we could show how important it is to remove the ergot sclerotia from the bulk cereals as early as possible. Although the modern milling technology is highly sophisticated in grain cleaning, the ergot powder could not be removed satisfactorily. Ergot sclerotia on the contrary could be removed efficiently. If abrasion of rye is reduced to a minimum in the postharvest systems, the amount of toxic ergot alkaloids can also be reduced to a minimum. The amount of ergot (ergot alkaloids and ricinoleic acid) correlates with the amount of the peripheral layers in the flour as the ergot abrasion adheres at the grain surface. Consequently, a peeling step could reduce the amount of ergot. Of course, this cleaning step could not be used for wholemeal flour if the peeled bran fraction is less than 2–3%.

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