Saccharides in Ergot Sclerotia

The saccharides of ergot sclerotia and of kernels of triticale harvested from the same heads from which the ergot sclerotia were obtained were quantitatively determined by gas–liquid chromatography. The saccharide compositions differed drastically. Ergot sclerotia contained trehalose, mannitol, arabinitol, and erythritol. Trehalose was the major saccharide followed by mannitol. Small sclerotia contained less mannitol than larger size sclerotia. Saccharides normally present in mature cereal grains—sucrose, raffinose, ketose, nystose, fructosylraffinose—which were found in triticale grain were not detected in ergot sclerotia.

Ergot is caused by fungi (*Claviceps* sp.) which attack cereals. Among these parasitic fungi, *Claviceps purpurea* is the outstanding species, forming its conspicuous sclerotial state in the heads of cereal grains (Bové, 1970).

Ergot is probably best known as a disease of rye, but it also affects wheat, barley, triticale, corn, rice, and other small grains. Actually no known commercially grown varieties of cereal grains are completely resistant to ergot (Lorenz, 1978).

Several soluble carbohydrates—glucose, trehalose, mannitol, arabinitol, inositol, and erythritol—have been detected in ergot sclerotia. Trehalose and mannitol are the major carbohydrates and the predominant components in the nutrition of the fungus (Cooke and Mitchell, 1969, 1970). The sugars are synthesized via a lipid-consuming system activated during low-temperature dormancy to support germination and stroma production. Ergot sclerotia show an increase in mannitol and trehalose during activation. Subsequently, the levels of these carbohydrates decline during germination to an apparently constant level (Cooke and Mitchell, 1969, 1970).

The roles of arabinitol, glucose, inositol, and erythritol are unknown. Arabinitol disappears during either dormancy or activation and does not subsequently reappear.

All the determinations of sugars and polyols in ergot sclerotia are qualitative in nature. Quantitative data are lacking.

In this study quantitative determinations were made of the saccharides of ergot sclerotia as well as of kernels of triticale from the same heads from which the ergot sclerotia were harvested. The saccharide contents of sclerotia varying in size were also compared.

MATERIALS AND METHODS

Sampling. Ergot sclerotia were separated from two semidwarf varieties of triticale which were heavily contaminated with ergot. Heavy ergot contamination, seldom seen in the dry climate of Colorado, was observed in 1976 on several semidwarf triticale selections, which originated in the CIMMYT program and were planted on experimental plots at the Colorado State University Agronomy Research Farm at Fort Collins, CO.

The triticales were harvested from one-acre plots. Grain yields of the two varieties were 35.3 and 24.0 bushels/acre. Test weights were 57.2 and 53.6 lb/bushel, respectively. After harvest 20-lb subsamples of grain from each variety were taken and ergot sclerotia were separated by hand.

Sugar determinations were first made on a composite from each sample. Thereafter, the largest and smallest intact sclerotia were separated from the sample, measured with a caliper, and weighed. The large sclerotia were 1.05 ± 0.12 cm long and had an average weight of 65.6 mg; the smallest sclerotia were 0.47 ± 0.05 cm long and had an average weight of 8.6 mg. Ten or more of each group were milled and assayed for sugar composition.

Since the saccharide composition of the sclerotia from the two varieties was very similar, data for only one of the two are presented.

Sugar Analysis. Ergot sclerotia and kernels of triticale were milled 45–60 s in a stainless steel ball mill. Weighed amounts of the powders were mixed with 70% ethanol (v/v) in screw-cap vials to give 100 mg/mL, heated to 70–75 °C for 1 h, cooled, and centrifuged. Aliquots of the supernatant were evaporated to dryness under nitrogen at 60 °C and silylated overnight at room temperature with shaking using Tri-Sil reagent. The saccharides were separated on a Hewlett-Packard 5830 A gas chromatograph using a flame ionization detector. The unit was equipped with a $^1/_8$ in. × 6 ft stainless steel column packed with 3% OV-1 on Chromosorb W (HP 80–100). It was temperature programmed from 100 to 300 °C at 20 °C/min (Becker et al., 1977). The saccharides were identified by cochromatography with authentic standards. Identity conformation was obtained by comparison with authentic standards using one or more of the following procedures where applicable: enzymatic hydrolysis, chemical hydrolysis, borohydride reduction. The sugars were quantitated by comparison of their GC peak areas with standard curves constructed using known amounts of saccharides. The reported results are averages of two or more separate determinations and agreed within 10%.

Autolysis. Weighed amounts of ball-milled ergot and triticale grain powders were mixed with 0.2 M phosphate buffer (pH 6.5) in screw-cap vials to make 100 mg/mL, 2 drops of toluene were added and the vials incubated at 37 °C for 16 h with continuous shaking. Triticale grain powders were also autolyzed in 0.2 M acetate buffer (pH 5.0). The samples were cooled to room temperature, centrifuged, and aliquots evaporated to dryness under nitrogen at 60 °C. Duplicate samples were assayed for their saccharide content by GLC as described above.

RESULTS AND DISCUSSION

(a) Comparison in Saccharide Content between Ergot Sclerotia and Triticale Grain. The saccharide compositions of ergot sclerotia and of kernels of triticale harvested from the same heads from which the ergot sclerotia were taken are presented in Table I. Trehalose and mannitol were the major saccharides before autolysis, which agrees with the findings of Cooke and Mitchell (1969, 1970). However, glucose, which was detected by Cooke and Mitchell (1969) using paper chromatography to separate ergot extracts, was not found in this study presumably due to differences in the hosts of the fungus (*Claviceps purpurea*). Cooke and Mitchell (1969) harvested the ergot sclerotia from *Phalaris arundinacea* (Reed Canary Grass), while in this study the sclerotia grew on triticale. Saccharides normally present in mature cereal grains—sucrose, raffinose, ketose, nystose, fructosylraffinose—were not detected in ergot sclerotia.

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Table I. Saccharides in Ergot Sclerotia and Kernels of Triticale (Percent, Dry Weight Basis)[a]

saccharide	ergot sclerotia before autolysis	ergot sclerotia after autolysis (pH 6.5)	triticale before autolysis	triticale after autolysis (pH 6.5)	triticale after autolysis (pH 5.0)
arabinitol	0.40	0.34			
erythritol	0.02	0.01			
glucose	0.00	9.41		n.d.[d]	n.d.
mannitol	0.60	0.49			
trehalose	3.90	0.00			
inositol	0.10	0.40	0.09	0.13	0.28
sucrose			1.70	0.00	0.00
maltose			0.00	1.41	1.45
trisaccharides[b]			1.71	0.40	0.14
tetrasaccharides[c]			0.21	0.11	0.07

[a] Data are averages of two or more separate determinations. [b] Raffinose and kestose. [c] Nystose and fructosylraffinose. [d] n.d. = not determined.

The saccharide composition was altered by autolyzing the sample as expected. Methods involving extracts with water or water–alcohol mixtures yielded different saccharide results in spite of precautions designed to prevent amylase action during extraction of samples of wheat for saccharide determinatins (D'Appolonia et al., 1971). Such methods provide an indication of indigenous saccharidase activity of a grain sample. It has, therefore, been suggested that in addition to water extracts other techniques be used to determine saccharide composition.

While there were only slight decreases in arabinitol, erythritol, and mannitol, trehalose disappeared completely and nearly stoichiometric amounts of glucose were detected. Autolysis produced higher amounts of inositol compared to extraction with ethanol. Conditions are more favorable for the action of phytase extracting the sample in the pH 6.5 buffer solution.

The saccharide composition of triticale grain, presented in Table I is very similar to those published by Becker et al. (1977) for different varieties of triticale. The absence of monosaccharides in triticale is consistent with observations on wheat grains by Meredith and Jenkins (1973a,b) and Täufel et al. (1959). Sucrose is the major sugar occurring in approximately the same amounts as the next abundant sugars raffinose and kestose combined, which is in agreement with previous results (Becker et al., 1977).

Incubation of the triticale sample in pH 6.5 and 5.0 buffer solutions, respectively, caused the indigenous enzymes to hydrolyze starch to maltose and sucrose to glucose and fructose. Autolysis produced higher amounts of inositol compared to extraction with ethanol. The largest amount was produced at pH 5.0, the optimum pH for phytase.

(b) Effect of Size of Sclerotia on Saccharide Composition. Sclerotia of a given *Claviceps* sp. vary in alkaloid content. The small sclerotia tend to have lower alkaloid contents than the larger ones harvested from the same host (Deufel, 1952; Silber and Bischoff, 1954), which was the reason for analyzing large and small sclerotia for saccharide composition. The data are given in Table II.

Table II. Saccharides in Ergot Sclerotia Varying in Size (Percent, Dry Weight Basis)[a]

saccharide	large sclerotia (av 65.6 mg)	small sclerotia (av 8.3 mg)
arabinitol	0.37	0.36
erythritol	0.03	0.02
glucose	0.00	0.00
mannitol	0.72	0.50
trehalose	3.81	3.83
inositol	0.10	0.11

[a] Data are averages of two or more separate determinations.

Only mannitol was significantly different comparing sclerotia of different sizes. Mannitol, however, is one of the major carbohydrates in the nutrition of the fungus (Cooke and Mitchell, 1969, 1970). This difference in mannitol content, therefore, might have some significance in the activation and germination of small compared to large sclerotia of ergot.

CONCLUSIONS

The saccharide composition of ergot sclerotia differs drastically from that of kernels of grain grown on the same head. None of the saccharides of triticale were detected in ergot sclerotia. Trehalose was the major saccharide in sclerotia, followed by mannitol which agreed with previously published data. Smaller sclerotia contained less mannitol than the larger ones.

LITERATURE CITED

Becker, R., Lorenz, K., Saunders, R. M., *J. Agric. Food Chem.* **25**, 1115 (1977).
Bové, F. M., "The Story of Ergot", J. Karger, Basel, Switzerland, 1970.
Cooke, R. C., Mitchell, D. T., *Trans. Br. Mycol. Soc.* **52**, 365 (1969).
Cooke, R. C., Mitchell, D. T. *Trans. Br. Mycol. Soc.* **54**, 93 (1970).
D'Appolonia, B. L., Gilles, K. A., Osman, E. M., Pomeranz, Y., in "Wheat Chemistry and Technology", American Association of Cereal Chemists, Inc., St. Paul, MN, 1971, p 313.
Deufel, J., *Naturwissenschaften* **39**, 433 (1952).
Lorenz, K., *Crit. Rev. Food Sci. Nutr.*, in press (1978).
Meredith, P., Jenkins, L. D., *N.Z. J. Sci.* **16**, 599 (1973a).
Meredith, P., Jenkins, L. D., *Cereal Chem.* **50**, 243 (1973b).
Silber, A., Bischoff, W., *Pharmazie* **9**, 46 (1954).
Täufel, K., Romminger, K., Hirschfeld, Z. *Lebensm.-Untersuch. Forsch* **109**, 1 (1959).

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