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Ribose in Meat

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Careful chromatographic methods for detection, estimation and quantitative determination of ribose in meat are described in detail. Samples of meat from the gracilis-muscle of hogs and cattle were collected and examined immediately after slaughter as well as after different kinds of storage up to 6 months.

Immediately after slaughter all samples of meat from cattle contained less than 5, probably less than 1 μg of ribose per g meat, that is, no ribose could be detected by the technique used which does not account for the ribose bound, *e. g.*, in ribonucleic acids in the samples. In a few samples of meat from hogs immediately after slaughter traces of ribose could be detected, but in most samples none.

During storage under refrigeration the ribose content of the meat, as determined by the methods described increases up to 120 μg per g meat in 16 days (meat from cattle). The increase is practically proportional to storage-time. Cold storage of meat (−20°C) shows the same result, so that a ribose content, defined as above, of 60 μg per g meat can be obtained after 1 month. If meat is frozen immediately after slaughter and then kept in cold storage, it will last much longer until ribose can be detected by the methods described.

For several reasons it seems likely that the ribose detected and determined by using the methods described is free or loosely bound.

Even very small amounts of ribose may be of importance in meat influencing its properties as a chemical system as well as a microbiological medium. Therefore it seemed to be of interest to know if "free" ribose is formed in meat from, *e.g.*, ribonucleic acids during ordinary handling and storage of meat, and if so, what quantities of ribose would be formed by different kinds of handling and storage. Possibly a method of determining "free" ribose would furnish a tool for the judging of meat in regard to certain qualities.

The term free ribose is used here for the quantity of ribose which can be detected by the mild methods developed, even if some part of this quantity might be formed, *e.g.*, from more complex products of hydrolysis of ribonucleic acids, such as mononucleotides, during analysis.

A similar investigation on deoxyribose in meat is beeing carried out, the results of which will be published later.

EXPERIMENTAL

Meat from cattle and hogs were used in this work. In order to get defined meat the raw material was obtained from the gracilis-muscle only.

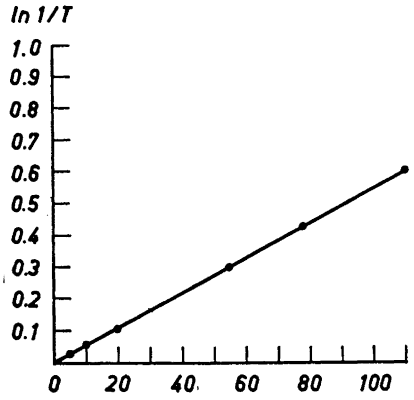

Fig. 1. Evaluation curve for ribose from light absorbance at 5 100 Å. Extinction in 10 mm cells.

First a method for detection and estimation of ribose was developed, then a method for the quantitative determination of ribose in meat was worked out. It is essential in both methods that free ribose can be extracted with no or the slightest possible hydrolysis of ribonucleid acids or other compounds containing ribose.

Detection and estimation of ribose in meat. To 30 g of finely but carefully ground sample of meat 110 ml of distilled water and 30 ml of 20 % phosphotungstic acid were added. The mixture was well stirred, then heated on a waterbath for 10 min and centrifuged. The waterphase was filtered into a glassvessel, desalted using Dowex 50, evaporated and made up to 5 ml.

Paper chromatograms were taken using the technique of Partridge [1] with modifications published later by several authors as described by Block *et al.*[2] On every chromatogram known quantities of ribose were run. As usual parallel chromatograms were carried out using other carbohydrates and related compounds which occur in meat.

Quantitative determination of ribose in meat. To 50 g of finely but carefully ground sample of meat 100 ml of distilled water and 50 ml of 20 % phosphotungstic acid were added. The mixture was then treated as described above under Detection and estimation, except that the desalted, evaporated filtrate was made up to 10 ml.

The chromatographic method, which was used by Pridham [3] for determination of aldohexoses, some aldopentoses though not ribose, hexuronic acids and 6-deoxy-aldohexoses was found to give excellent results with ribose too and therefore was used.

After the colour of the spots had been eluted from the paper with aqueous methanol containing stannous chloride (1.0 g of stannous chloride in 5 ml of water + 90 ml of absolute methanol, then filtered) the absorbances were measured in a Zeiss spectrophotometer PMQ II at 5 100 Å. At this wavelength there is an absorption maximum for the coloured compounds formed by the interaction of ribose with the *p*-anisidine-hydrochloride reagent.

The same precautions to avoid confusion were observed, as were described above under Detection and estimation. The evaluation curves obtained with known quantities of ribose on the same paper chromatograms, using the same procedure, application, development, drying, spraying with *p*-anisidine, eluting and spectrophotometric measuring show straight lines with varying amounts of ribose from 5 to 110 μg as will be seen from Fig. 1.

RESULTS

Detection of ribose in meat. Samples of meat from cattle were collected:

1. Immediately after slaughter, that is within 15 min.
2. After storage under refrigeration (+ 1 to + 4°C) for 2, 6, 8, 10, 13 and 15 days.

Table 1. Chromatography of free ribose in meat from cattle. Ribose spots.

Time Days	1 No storage	2 Storage under refr.	3 Cold storage	4 Immediately frozen, then cold storage	5 Kept minced with water	6 Kept minced with water +phospho-tungstic acid
0	None	—	—	—	—	—
1	—	—	—	None	—	—
2	—	Trace	—	—	Trace	None
3	—	—	—	None	—	—
6	—	Defined spot	—	—	Defined spot	None
7	—	—	—	None	—	—
8	—	Defined spot	—	—	Defined spot	None
10	—	Defined spot	—	—	Strong spot	None
13	—	Strong spot	—	—	Strong spot	None
14	—	Strong spot	—	None	—	—
15	—	Very strong spot	—	—	Very strong spot	None
Months						
1	—	—	—	None	—	—
2	—	—	Defined spot	—	—	—
6	—	—	Defined spot	—	—	—

Table 2. Chromatography of free ribose in meat from hogs. Ribose spots.

Time	1 No storage	2 Storage under refr.	3 Cold storage
0	None to traces	—	—
2 days	—	Defined spot	—
8 days	—	Strong spot	—
4 months	—	—	Defined to strong spots

3. After 2 and 6 months cold storage at −20°C. Carcasses handled commercially before cold storage, including refrigeration.
4. Samples collected within 15 min after slaughter, then immediately frozen (within 1 h after slaughter), then kept 1, 3, 7, 14 and 30 days at −20°C.
5. Samples collected and ground within 1 h after slaughter, then 110 ml dist. water added to 30 g ground meat. The mixture kept under refrigeration (+ 1 to + 4°C) during 2, 6, 8, 10, 13 and 15 days.
6. The same series as sub 5 but 110 ml dist. water + 30 ml 20 % phosphotungstic acid added instead of water only.

Samples of meat from hogs were collected as follows.
1. Immediately after slaughter.
2. After storage under refrigeration (+ 1 to + 4°C) for 2 and 8 days.
3. After cold storage for 4 months at −20°C.

Tables 1 and 2 contain the results of qualitative chromotogrophy.

Quantitative determination of ribose in meat. Table 3 gives the results of the quantitative measurements of ribose in meat from cattle determined as described above. Samples were collected, 1) Immediately after slaughter 2) After storage under refrigeration (+ 1 to + 4°C) for 2, 4, 6 and 16 days, 3). After cold storage at −20°C during 1 month. 0.02 ml of resulting solution applied.

Table 3. Quantities of free ribose in meat from cattle after different handling.

Samples	Number of samples	Ribose of spot μg
Immediately after slaughter	10	None
Refrigerated for:		
2 days	3	1
4 days	1	8
6 days	4	5−7
16 days	1	12
Cold storage for:		
1 month	3	6

CONCLUSIONS AND DISCUSSION

Whilst ribose cannot be detected in fresh meat from cattle immediately after slaughter, and only in traces and in some cases of such meat from hogs if the careful chromatographic methods described are used which allow detection of about 1 μg of ribose, there will appear an increasing amount of ribose *e.g.* during storage even if this storage is carried out under refrigeration or as cold storage. Immediate freezing of the meat after slaughter delays the appearence of ribose for at least 1 month.

Though it is not possible at present to decide if the quantity of ribose detected and determined by the methods described exists as free ribose or as partly bound, there are reasons to believe that this quantity of ribose is in a free or loosely bound state. The most important reason to think so seems to be the fact that all samples examined have been very finely ground in order to allow all chemical compounds therein to be uniformly distributed in the samples and free to be attacked by chemical action during analytical procedure.

REFERENCES

1. Partridge, S. M. *Nature* **164** (1949) 443.
2. Block, R. J. Durrum, E. L. and Zweig, G. *A Manual of paper chromatography and paper electrophoresis.* New York 1958, p. 170−188.
3. Pridham, J. B. *Anal. Chem.* **28** (1956) 1967.

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