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QUANTITATIVE AND QUALITATIVE DETERMINATION OF CONNECTIVE TISSUE CONTENT OF MEAT AND MEAT PRODUCTS.

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We can accept the statements in the literature /1, 2, 3 etc./ that a close connection exists between the tenderness of meat and the quantity of connective tissue contained in it. This is why an accurate quantitative and qualitative determination of the connective tissue content /collagen, elastin/ is of a considerable importance when grading meat and meat products.

Assuming that the tenderness of sliced or large pieces of raw meat has not been influenced artificially /e.g.by enzymes/ it is possible to form an view of the connective tissue content, that is to say, on the tenderness of the meat by applying the penetrometer, or shear-force principle /4, 5, 6/. With finely minced meat this simple procedure is not usable. For certain products of special quality, primarily for the Hungarian salami, - well known also abroad -, our standards determine strictly and also quantitatively the tolerance of connective tissue contents /7/. The said product must not contain more than 1,5 per cent. of macroscopically isolable tendon and connective tissue. Pieces of connective tissue and /or tendon in the filling must not exceed a length of 6 mm and their surface extension must not be more than o,18 sq.cm. This requirement is simply complied with by fine chopping. This is why we had to study the problems of accurate determination of the connective tissue contents of finely chopped meat.

Some of the published processes /Neumann and Logan /8/, Lampit, Baker and Brown /9/, Möhler and Antonacopoulos /1o/ transform collagen by autoclaving into water soluble gelatine and after acid hydrolysis of the gelatine and of the elastin, determine the hydroxyproline or glycocol contents of these

substances. In comparison with these tedious processes requiring a somewhat intricate equipment, the Striegel method /12/ modified by Lindner and Patschky /11/ is simpler and more suitable for practical purposes. It has, however, the shortcoming, that it permits the determination of collagen content only. In comparison the enzyme method of Grau and Hamm /13/ is, as shown by our comparative tests, also simple, giving more rapid and satisfactory results, though this method determines together collagen and elastin content. While the process of Grau and Hamm is suitable for meat, containing no fat, it cannot be used for testing finely chopped fatty meat or meat products, e.g. salami fillings. Several research workers /Lilienthal and Zierler /14/, Lowry, Gilligan and Katerssky /15/ made use of the insolubility of collagen and elastin in diluted alkali for isolating the supporting tissues, then they separated the collagen from the elastin by autoclaving and were so able to determine the latter separately too. With these latter processes the authors did not take into account that under certain conditions in diluted alkali the solution of connective tissues cannot be ignored and this will produce errors. A relatively simple and rapid method for practical purposes is arrived at, if the extent of the error, caused by alkaline solution is known and, the method is modified so as to reduce this error to a minimum.

Our tests have been carried out in the following manner: the meat /filling/ is ground twice on a 2 mm plate, and the connective tissue elements twisted around the knife, are carefully collected, finely chipped with shear and added to the comminuted mass. 2 - 2,5 g of the homogenized material are used for determining the total N by the usual Kjeldahl method. For determining the connective tissue N, 1,0 g of the material is weighed into a 100 ml test tube, 50 ml of 0,05/n alkali is added, the test tube is filled up with distilled water to the mark, the tube is kept for 24 hours - during which it is shaken several times - at room temperature and finally filtered. 50 ml/0,5 g/ of

the filtrate is heated with sulphuric acid and by the alkali extracted non-connective tissue N is determined by the Kjeldahl method. The connective tissue N is calculated from the two values by means of the following formula:

$$100 - \frac{\text{N soluble in meat}}{\text{total N}} \cdot 100 = \text{connective tissue N as}$$

percentage of the total N.

Accordingly only two N determinations are required for determining the connective tissue N. The following conclusions could be drawn from the series of tests made by this method:

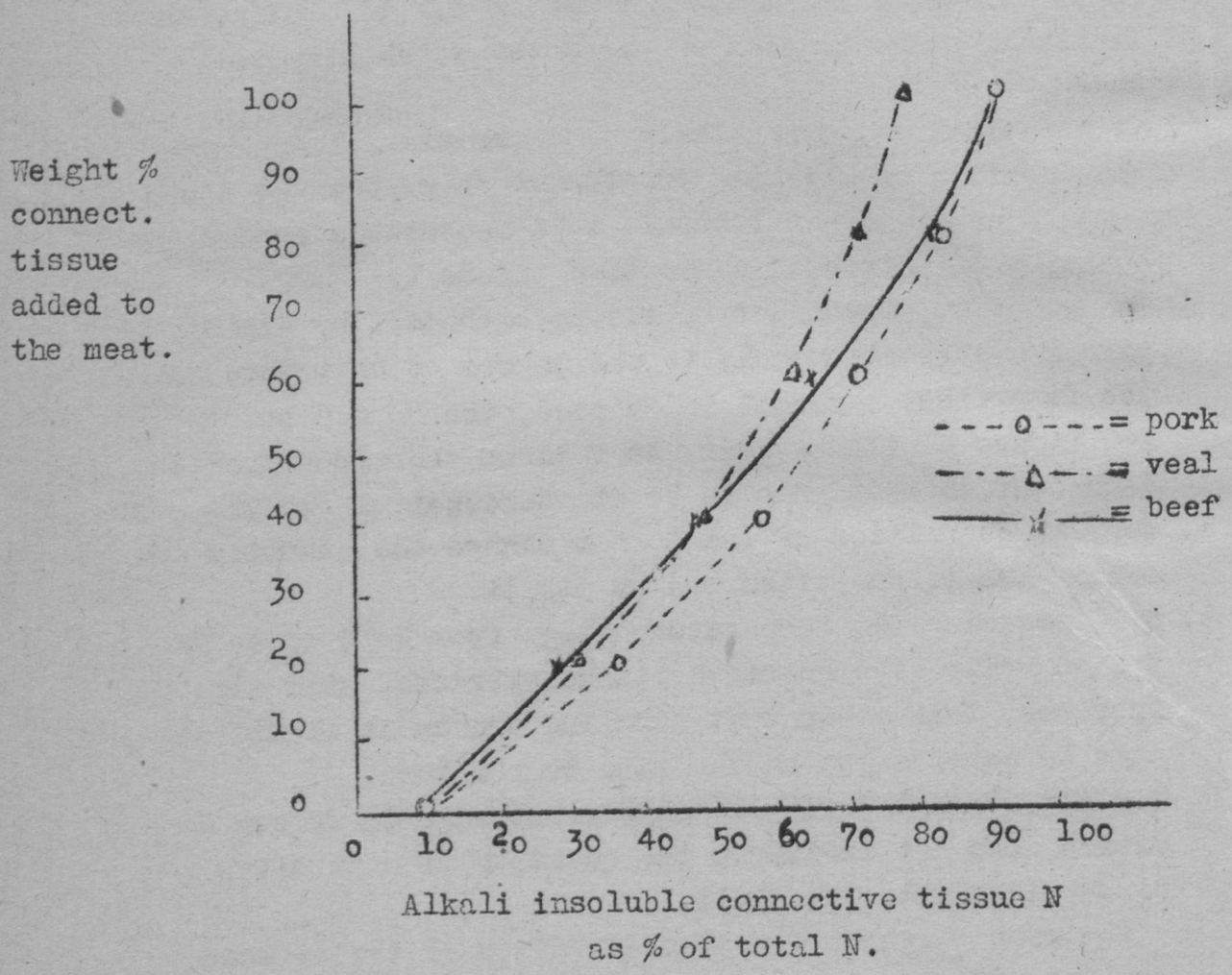
1. The solubility of the connective tissue does not change in the alkali concentration range of 0,1 to 0,01/n. Above an alkali concentration of 0,1/n the solubility will increase.
2. At identical /0,05/n/ alkali concentration, increasing the duration of alkaline treatement /e.g. from 24 hours to 30 hours/ did not increase to a measurable extent the quantity of extracted connective tissue N.
3. The quantity of connective tissue extracted by alkali increase proportionately to the degree of chopping, i.e. the finer the material is chopped, the more connective tissue N will be lost. It follows therefrom that the degree of chopping has to be standardized for the process.
4. Varying quantities of fat do not change the quantity of connective tissue extracted by alkali.
5. By increasing the temperature /e.g. from 20°C to 40°C/ the quantity of connective tissue extracted will also increase. This means that alkaline treatment should always be carried out at the same temperature.
6. Cooling storage during 14 days or storage in frozen condition does not influence the quantity of connective tissue insoluble in alkali.

In order to check the practicability of the process, we added to finely ground meat, - from which the visible connective tissue contents had carefully been trimmed -, different quantities of connective tissue which had

carefully been freed from muscle and fat tissue. As a result we found that the quantity of connective tissue insoluble in diluted alkali had increased in proportion to the percentage of connective tissue added to the meat /fig. No.1/.

Fig. No.1

Correlation between the weight percentage of connective tissue added to meat and the quantity of connective tissue insoluble in alkali.



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We weighed o,2, o,4, o,6, o,8, 1,o g of beef, Achilles-tendon into a loo ml test tube and determined therefrom the quantity of connective tissue insoluble in alkali, in order to check our process and the accuracy of the determination. Similarly we determined the quantity of connective tissue insoluble in alkali of o,2 - 1,o g of meat completely freed from visible connective tissue and, finally from a mixure of meat and tendon tissue, containing o,2, o,4 g etc. of tendon. The results are shown in table No.I.

Table No.I

Determined alkali insoluble connective tissue contents of trimmed tendon, trimmed meat and mixture of meat and tendon compared with calculated values.

Tendon or meat weighed in g/loo ml	Total N mg/loo ml		Alkali insoluble connective tissue N mg/loo ml				Tendon+meat weighed in mg/loo ml	Alkali insoluble connective tissue N mg/loo ml	
	tendon	meat	tendon meas.	tendon calc.	meat meas.	meat calc.		measured	calculated
o,o	o,o	o,o	o,o	o,o	o,o	o,o	o,o +1,o	6,7o	6,8o
o,2	16,8	6,94	14,9o	15,4	o,39	o,49	o,2 +o,8	38,97	38,81
o,4	33,6	13,89	3o,45	3o,84	1,o9	o,94	o,4 +o,6	58,93	59,2o
o,6	5o,4	<o,83	46,o	46,<7	1,63	1,41	o,6 +o,4	73,<5	73,43
o,8	67,<	27,77	61,6	61,69	1,99	1,9o	o,8 +o,2	83,62	83,86
1,o	84,o	34,72	76,95	77,11	2,32	2,36	1,o +o,o	91,61	91,8o

The date of the table show that the process is giving well reproducible results for practical purposes. We have determined the connective tissue contents of several half and finished products of the Hungarian Meat Industry having various qualities - these results also agree with our assumptions, that is to say e.g. undergrade meat pulp, dessert salami containing also beef, dry /Csaba/ sausage, the inferior quality of which could also be judged organoleptically, showed in each case higher contents of alkali insoluble connective tissue. Some of our very many determinations are shown in table No.II.

Table No. II.

Connective tissue N contents of different qualities of meat pulp and of sausages

Tested product	Total N mg/g	Alkali soluble connective tissue N mg/g	Alkali insoluble connective tissue N in % of total N
I class meat pulp	25,45	2o,3o	2o,3o
II class meat pulp	21,56	15,4o	28,6o
Dessert salami /containing 2o % beef/	4o,5o	34,3o	14,5o
Winter salami /made of pure pork/	29,1o	27,o2	7,2o
I class /dry/Csaba/ sausage	24,36	21,5o	12,35
II class /Csaba/ sausage	22,5o	18,2o	19,oo

It should be added to the data shown in the above tables that they practically tally with the data of Möhler and Antonacopoulos /1o/.

In order to classify some meat on basis of its chemically detectable connective tissue contents, it is naturally absolutely necessary to know the connective tissue contents of the muscle groups having anatomically different location of animals of differend kind, age and nutrition. We ourselves have also carried out detailed examinations in this respect; in order to save space we have not enumerated the data of these examinations: they are well known from the papers of other research workers. It is, however, worth while mentioning from our data that we always found larger connective tissue N quantities with young animals on the one hand and, with undernourished animals of the same kind and age on the other hand. We give a few data in this respect in table No.III /below/.

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Table No. III.

Connective tissue contents of the longissimus dorsi muscle from young and old cattle and ~~those~~ of high and low grade.

Description of animal examined	Total N mg/g	Connective tissue N mg/g	Connective tissue N as % of total N
3 weeks old calf Ist class	33,3	3,55	9,5o
3 weeks old calf IInd class	3[illegible],4	3,8o	11,4o
3 years old cow IInd class	35,1	2,9o	8,28
3 years old cow IIIrd class	32,7	3,9o	12,2o
5 years old bull Ist class	35,2	2,62	7,4o
5 years old bull IIIrd class	37,[illegible]	5,o1	13,45

The data of the above table are in support of our statement above, that the animals with better grades hase less connective tissue than the lower grades, and the young animals may have more connective tissue N than the older ones. It follows from this that it is not the quantity of connective tissue which is decisive for the chewing /cutting/ resistance of meat but the quality of such connective tissue. In this respect we may refer /in addition to our own histological observations/ to observations of Hiner, Anderson and Feller /16/ of a similar character. According to these the tissue fibres of older animals are thicker, more twisted in their run, unequal, more coarsely woven.

For practical purposes connective tissue consists ~~in~~ the main of kinds of protein which can be separated from each other: collagen and elastin. Various processes have been used for separating these two substances. We ourselves filter the connective tissue material insoluble in dilute alkali, wash it till free from alkali and then boil it together

with the filter-paper for 1 hour in 3o ml of distilled water. Thereafter we add o,2 g of tartaric acid, continue boiling for another 1/2 hour, finally wash it together with the filter-paper in a 5o ml test tube and fill it up to the mark. Of the substance thus obtained the N content of the filtrate is the collagen N, the N content of the non-extracted part is the elastin N. A few representative data of many examinations made by us in this manner are shown in table No. IV.

Table No. IV.

Collagen and elastin N contents of various connective tissue parts of animals of different kind and age

Denomination of supporting ligament	Total N mg/g	Collagen N mg/g	Elastin N mg/g	Collagen N as per cent. of total N	Elastin N as per cent. of total N
Achilles tendon of beef	62,1o	59,5	1,4	94,o	2,2
Achilles tendon of veal	58,8o	53,9	1,3	91,6	2,2
Musculus teres of beef	75,6o	14,o8	58,1	18,6	76,8
Musculus teres of veal	73,36	12,4	59,5	16,9	8o,3
Epi-and perimysium of shank of beef	78,9	57,7	11,1	73,1	14,o
Epi-and perimysium of shank of veal	61,3	49,9	8,12	81,5	13,5
Epi-and perimysium of shank of pork	54,6	49,5	o,91	88,8	1,66
Aorta of beef	41,7	9,4	18,5	22,5	44,3
Aorta of veal	4o,6	8,75	2o,65	21,5	5o,8
Aorta of pork	43,o	9,62	23,45	22,3	54,5

The data of the table IV show that connective tissues of pork contain considerably less elastin than those of beef, and that certain tissues of young animals contain more of it than those of old animals of the same kind. A young animal - assuming the same anatomical location - contains in its muscles generally more of these tissue-elements than older animals. The data shown in table No.IV and other examinations made by us also show that meat products, containing much tendon /tendon pulp/ and

trimmings, contain much collagen N while sausages /containing many blood-vessels and tendon-ends/ contain more elastin N.

By our process which can be further developed to some extent both methodically and also from a didactic point of view, the ratio of the collagen and elastin content of the different tissue elements can be well demonstrated /see Fig. No.2./

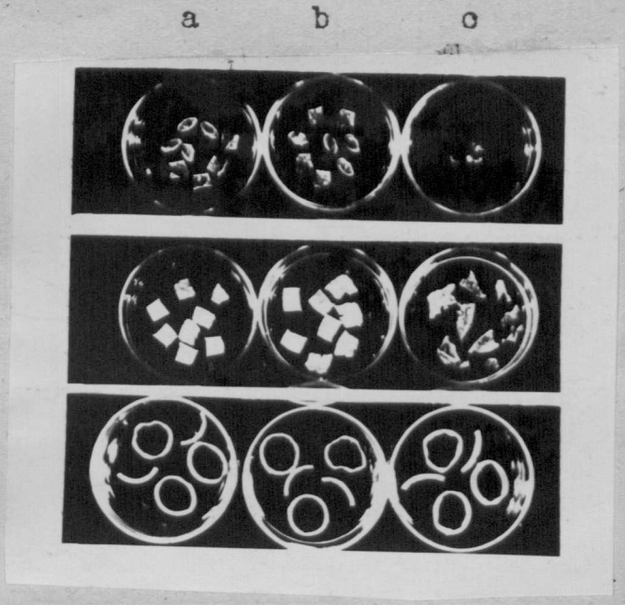

Fig.No.2 - Frozen histological section - A./ Beef Achilles tendon - B./ Beef ligamentum teres - C./ Beef aorta. - The first column /a/ shows the raw sections, /b/ the same sections after treatement in diluted alkali, the third column /c/ shows the sections after boiling in the tartaric acid solution.

The photographs shown in Fig. No 2 demonstrate the data given in table No. IV : the beef Achilles tendon hardly contains any elastin /A/c/, whereas the lig. teres of beef consists mainly of elastin /B/c/.

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Summary:

After a survey of the literature the authors describe a simple and rapid process for the qualitative and quantitative determination of connective tissue in meat and meat products. The purpose of the process is to determine the total N content of the meat and then to determine the quantity of connective tissue N insoluble in o.o5/n alkali.

The portion insoluble in alkali is washed with destilled water on filter-paper till it contains no more alkali and then boiled for an hour. Thereafter the substance is boiled for another 1/2 hour with tartaric acid and filtered. The filtrate indicates the quantity of collagen, the quantity of elastin can be determined from the non dissolved portion.

The sources of error of the process, their prevention and the reliability of the standard process are shown by series tests.

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Errata

ad Lőrincz-Szeredy' paper

Page, Paragr, Line	Error	Correct
2 1 4	chipped	chopped
5, 1st line in title of Table:	sontents	contents
6, text in 3rd column of Table:	Alkali soluble connective tissue N	Alkali soluble tissue N
7 2 1-2	in the main of kinds	in the main two kinds
8, in text of 1st column of Table:	Musculus teres	Ligamentum teres