SIMULTANEOUS DETERMINATION OF CONSTITUENTS OF MEATS AND MEAT PRODUCTS ON ONE SAMPLE

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Further improvement in the quality of meats and meat products is fundamentally determined by the results of the acceleration in meat research during the 197o-s. Besides the classical parameters for the biological value of meats and meat products, it is nowadays indispensable to know the meat protein content, the relative connective tissue protein content, the PER value, the foreign water content, the Feder number, the P number and the fat-protein ratio /1/.

Various automatic equipment and procedures are available for the serial analysis of meat products. However, the rapid introduction of expensive automatic procedures and instrumentation in almost half a million low-capacity meat plants is not probable, particularly in the developing countries. Taking into consideration the demands of such small plants too, we set out to develop a manual analysis procedure which would satisfy the following conditions: /1/ it should permit the simultaneous analysis of the meat components on one sample by elementary analytical means; /2/ the procedure should be relatively fast; /3/ the procedure should not be expensive; /4/ the procedure should allow the full quality control of meats and meat products at an up-to-date level.

It has been demonstrated by Körmendy et al. /2/ that, when heated at 18o°C, meats and meat products undergo dehydration within 2o minutes. We carried out experiments to prove that heating at 18o°C does not cause fat or protein decomposition. Measurement of the fat content was begun with liquid-liquid extraction: aqueous hydrochloric acid solution and sulphuric acid solution was used with and without a tin /II/ chloride catalyst to liberate the fats. The excellent, non-toxic fat solvent tetrachloroethylene was employed for extraction. As control, the Weybull-Stoldt procedure was utilized /3/. The experimental results are listed in Table 1. It can be stated that in none of the measurement alternatives did the preliminary dehydration at 18o°C cause a significant difference in the fat contents of the samples as compered to the control. At the reliability level P = o,o5, the calculated F values were lower in all experimental modes than the tabulated F values.

These aqueous mineral acid solutions were deliberately chosen for the study of changes in the fat content: after hydrolysis for about 15 hours, the 4-hydroxyproline content of the sample can be determined on the defatted aqueous acidid solution, and from this the connective tissue protein content of the meat or meat product can be calculated /8/. Subsequently, the samples were hydrolyzed only with 3o % sulphuric acid solution in the presence of tin/II/ chloride as catalyst. The meat protein content, the connective tissue protein content, the calcium and the total phosphorus contents were determined on aliquots of the defatted acidic solution.

Table 2 shows the meat protein contents of meats and meat products dehydrated at 18o°C. These contents were determined with zirconium/IV/ oxide catalysis /9/. It may be said that the 18o°C dehydration did not cause any protein loss; the difference of the various measurement series from one another is not significant, and the calculated F values are lower at a reliability level of p = o.o5 than the tabulated F values. For determination of the calcium content, we also set out from the defatted acidic solution. Decomposition was achieved with zirconium/IV/ oxide as catalyst, and the calcium determined on the resulting solution via a known method.

For total phosphorus content determination, an aliquot of the defatted acidid solution was decomposed with selenium-mixture catalysator. Phosphorus was measured colorimetrically on the resulting solution, with ammonium molybdate - metavanadate.

The connective tissue protein content was determined on the residual defatted acidid solution from which the aliquots had been taken for protein, calcium and phosphorus measurements. Hydrolysis was continued for a further 15 hours at 1o5°C. The use of sulphuric acid allows both the fat liberation and the hydrolysis to be performed without danger in a drying oven. The 4-hydroxyproline content of the hydrolyzate was measured colorimetrically, with p-dimethylaminobenzaldehyde as reagent.

The course of the simultaneous analysis:

/1/ Dehydration

5 g accurately weighed sample of homogenized meat or meat product is heated at 18o°C in a Petri dish in a drying oven. After cooling, it is reweighed.

$$\text{Water content /\%/} = \frac{G - G_1}{G} 1oo$$; G: mass of sample taken /g/; G_1: mass of dried sample /g/.

/2/ Fat content determination

The dehydrated sample is washed with a little 3o% sulphuric acid in a 25o cm³ flask. Further 3o% sulphuric acid is added so that a total of 5o cm³ is used. Before these operations 1 g $SnCl_2.H_2O$ is dissolved in the 5o cm³ 3o% sulphuric acid. The acidic solution is refluxed for 1o minutes, and 1oo cm³ tetrachloroethylene is then added at 6o°C. Refluxing is continued under stirring for 1o minutes. After cooling, the two phases are separated in a separating funnel: the lower, solvent phase is run off into a 2oo cm³ volumetric flask. The above extraction is repeated with 95 cm³ tetrachloroethylene, the extracts are combined, and the total volume is made up to 2oo cm³ with tetrachloroethylene. 2o cm³ aliquots are taken from the resulting stock solution. After drying under an infrared lamp with the use of an exhaust, the residual material is dried to weight constancy at 1o5°C.

Fat content/%/ $= \frac{lo/G_1-G_o/}{G}$ loo; G_1:mass of Petri dish+fat/g/; G_o:mass of Petri dish/g/; G:mass of sample before drying/g/.

/3/ Meat protein content determination

The aqueous acidic phase obtained in point 2 is placed in a 25o cm³ beaker and boiled at loo°C until the small amount of residual solvent has evaporated /ca. lo minutes/. The solution is then washed into a loo cm³ volumetric flask, and the volume is made up to loo cm³ with 3o% sulphuric acid. 2o cm³ of the resulting stock solution is pipetted into a 25o cm³ Kjeldahl flask, and exactly 3o cm³ concentrated sulphuric acid, lo g K_2SO_4, o.6 g $CuSO_4.5H_2O$ and o.2 g ZrO_2 are added. The solution is boiled until it becomes clear, when it is pale bluish-green. The decomposition time is 15-4o minutes, depending on the protein content of the sample.

The clear solution is washed into a 2oo cm³ volumetric flask, the volume is made up to the mark with water, and the quantity of ammonia formed from a 2o cm³ aliquot is determined with a Parnas-Wagner apparatus.

/4/ Connective tissue protein content determination

The residual 8o cm³ from the aqueous acidid stock solution preparad in point 3 is heated at lo5°C for 15 hours. /It is convenient to leave it to stand overnight in a drying oven at lo5°C./ The hydrolyzate is washed into a 2oo cm³ volumetric flask, and the volume is made up with distilled water. The resulting solution is filtered through a fluted filter and lo cm³ of the filtrate is pipetted into a beaker. The pH of the solution is brought to 4 with 25% NaOH, and then to 8 with o.1 N NaOH solution. The neutralized solution is washed into a 2oo cm³ volumetric flask, and the volume is made up with distilled water. After standing for 1 hour at room temperature, the $Sn/OH/_2$ precipitate separating out is filtered off. 2 cm³ of the filtrate is transferred to a test-tube, 1 cm³ Chloramine-T solution is added /1.4o g Chloramine-T is dissolved in a mixture of 25 cm³ distilled water and 25 cm³ n-propanol in a loo cm³ volumetric flask, and the volume is made up with citrate.acetate buffer/, the solution is shaken and left to stand for 2o minutes at room temperature. Next, 1 cm³ of the colour-forming reagent solution is added /lo g p-dime thylaminobenzaldehyde is dissolved in 35 cm³ 6o % aqueous perchloric acid solution, and 65 cm³ isopropanol is added/. After being shaken, the mixture is heated in a 6o°C water-bath for 3o minutes, and after cooling, corolimetry is performed at 56o nm. Evaluation is made by comparison to the concentrations of a known solution series. Preparation of calibration curve: 5o mg hydoxyproline is dissolved in 5o cm³ distilled water containing 1 cm³ o.1 N hydrochloric acid in a loo cm³ volumetric flask, and the volume is made up with distilled water. 5 cm³ of this hydroxyproline stock solution is diluted to 5oo cm³ with distilled water. lo, 2o, 3o, 4o and 6o cm³ aliquots of this solution are transferred to loo cm³ volumetric flasks, and the volumes made up with distilled water. 2 cm³ is taken from each of these diluted solutions, oxidized for 2o minutes with 1 cm³ Chloramine-T solution, and 1 cm³ colour-forming reagent solution is added to the mixture, which is kept in a 6o°C waterbath for 3o minutes. In this case too colorimetry is performed at 56o nm.

/5/ Calcium content determination /lo/

/6/ Total phosphorus content determination

The aqueous acidid solution obtained after fat determination /see point 2/ is used for determination of the total phosphorus content. 2o cm³ of the loo cm³ defatted stock solution /see point 3/ is transferred to a 25o cm³ Kjeldahl flask. In turn, lo g K_2SO_4, o,5 g Se-mix catalyst /Se-Mischung, Merck/ and 2o cm³ concentrated sulphuric acid are added to the solution. Boiling is continued until the solution clears. The decomposition time varies between 15 and 35 minutes, depending on the protein content of the sample. After cooling, the solution is diluted to 2oo cm³ with distilled water. 5 cm³ of the solution is pipetted into a 2o cm³ volumetric flask, and 2.5 cm³ phosphorus reagent is added /2o g ammonium molybdate tetrahydrate is dissolved in 4oo cm³ distilled water, a solution of 1 g ammonium metavanadate in 4oo cm³ distilled water is added, followed by 14o cm³ concentrated nitric acid, and the volume is made up to looo cm³/. The volume is made up with distilled water, and the mixture is heated for lo minutes on a water-bath. The phosphorus content of the solution is determined colorimetrically at 42o nm, simultaneously with the colorimetry of a comparative solution.

Table 3 gives the results of analysis according to points 1-6 of pork, beef, separated meat, sausage types and Bologna sausage.

By simplification of the procedure detailed in points 1-6, a rapid analytical method was developed. The elements of this: /1/ Sampling, preparation of homogenizate, weighing of sample. /2/ Dehydration at 18o°C. /3/ Reweighing, calculation of water content. /4/ Boiling of dry residue with 2o-fold volume of tetrachloroethylene for lo minutes. The fat-containing extract is decanted off, and the dry residue is freed from solvent at loo°C for 1-2 minutes. /5/ Reweighing, calculation of fat content, meat protein and mineral matter contents.

Correction factors for calculation of protein contents: pork meat:o,95; beef meat: o,93, separated meat /pork/: o,87; sausages: o,82; Bologna sausage: o,88.

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Table 1. Determination of fat contents of meats and meat products dehidrated at 18o°C

	Stegeman-Stalder /a/		ISO /b/		Wyler /c/		Möhler-Volley /d/		Control /e/	
	Fat content %									
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Pork /Musc.long.dorsi/	1,733	o,127	1,743	o,o51	1,723	o,o31	1,787	o,o5o	1,713	o,o15
Beef /Musc.long.dorsi/	7,64o	o,o44	7,673	o,o8o	7,57o	o,o46	7,67o	o,o4o	7,717	o,155
Separated meat /From mixed pig bones/	46,247	o,131	46,7oo	o,175	46,6oo	o,3o5	46,733	o,123	46,697	o,111
Sausage I.	34,163	o,126	34,183	o,17o	34,44o	o,233	34,36o	o,175	34,53o	o,o61
Sausage II.	46,o63	o,122	45,933	o,o85	45,957	o,155	45,987	o,163	46,o33	o,o55
Bologna sausage	21,233	o,215	21,177	o,16o	21,32o	o,125	21,o7o	o,o66	21,4oo	o,o95

/a/ 4 g homogenizate, loo cm³ 6 N HCl /4/

/b/ 4 g homogenizate, loo cm³ 5 N HCl, 1,5 g $SnCl_2 \cdot H_2O$ /5/

/c/ 4 g homogenizate, 3o cm³ 3o % H_2SO_4 /6/

/d/ lo g homogenizate, loo cm³ 3o % H_2SO_4, 2 g $SnCl_2 \cdot 2H_2O$ /7/

/e/ control, Weybull - Stoldt procedure /3/

Table 2. Determination of meat protein contents of meats and meat products dehidrated at 18o°C

	I.		II.		III.	
	Meat protein content %					
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Pork /Musc.long.dorsi/	21,863	o,o81	21,88o	o,o36	21,9o7	o,o64
Beef /Musc. long.dorsi/	2o,117	o,o4o	2o,14o	o,o66	2o,193	o,o91
Separated meat /From mixed pig bones/	11,137	o,112	11,197	o,o76	11,163	o,o72
Sausage I.	16,527	o,126	16,24o	o,o79	16,263	o,o76
Sausage II.	25,437	o,o74	25,37o	o,122	25,513	o,1oo
Bologna sausage	9,o5o	o,o89	9,o8o	o,o7o	9,o8o	o,o5o

I. dehidrated sample ZrO_2 catalysis

II. not dehidrated sample ZrO_2 catalysis /control/ /9/

III. not dehidrated sample $CuSO_4$ catalysis /control/ /9/

Table 3. Analysis of meats and meat products on one sample

	Pork /Musc.long. dorsi/		Beef /Musc.long. dorsi/		Separated meat /From mixed pig bones/		Sausage I.		Sausage II.		Bologna sausage	
	I.	II.	I.	II.	I.	II.	I.	II.	I.	II.	I.	II.
Water content /%/	74,37	74,48	7o,82	69,96	43,1o	43,25	45,16	45,24	23,12	22,61	64,63	65,oo
Fat content /%/	1,79	1,71	7,67	7,72	46,73	46,7o	34,36	34,53	45,98	46,o3	21,o7	21,4o
Meat protein content%	21,86	21,88	2o,12	2o,14	11,14	11,2o	16,53	16,24	25,44	25,37	9,o5	9,o8
Connective tissue protein content %/	1,15	1,2o	2,8o	2,75	2,16	2,21	4,35	4,2o	5,9o	6,oo	1,4o	1,4o
Meat protein content free from connective tissue protein /%/	2o,71	2o,68	17,32	17,39	8,98	8,99	11,98	12,o4	19,54	19,37	7,65	7,68
Relative connective tissue protein content /%/	5,26	5,48	13,92	13,65	19,39	19,73	26,32	25,86	23,19	23,65	15,47	15,42
Feder number	3,4o	3,4o	3,5o	3,47	3,87	3,86	2,73	2,79	o,91	o,89	7,14	7,16
Foreign water content %	-	-	-	-	-	-	-	-	-	-	28,43	28,68
Fat-protein index	o,o8	o,o8	o,38	o,38	4,19	4,17	2,o8	2,13	1,81	1,81	2,33	2,36
P number	2,3o	2,3o	2,28	2,28	-	-	2,5o	2,54	2,25	2,26	4,oo	4,o4
Ca content /%/	o,o2	o,o2	o,o1	o,o1	o,o8	o,o9	o,o4	o,o4	o,o6	o,o6	o,o2	o,o2
Total phosphorus content /%/	o,22	o,22	o,2o	o,2o	-	-	o,18	o,18	o,25	o,25	o,16	o,16

I. Simultaneous procedure /mean values, n = 3/

II. Control /mean volues, n = 3/ analyzed on separate samples, without dehidration