Rapid Determination of Collagen in Meat Products

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SUMMARY: A method for hydrolyzing proteins by means of a microwave equipment was tested for its suitability to determine collagen and was compared with the hydrolysis method usually used for routine analysis by the Institute for Meat Hygiene. Materials of investigation were raw beef, ham, sausages, spread, gelatine and mixtures of gelatine with meat products. The microwave hydrolysis was carried out according to the instructions of the producer, the photometric determination of hydroxyproline by ISO-Method 3496.2. The comparison of the results obtained by the described techniques shows no statistically significant differences. The mean values of the recoveries are 102.1% (standard procedure) and 101.6 (microwave hydrolysis). The investigation shows that the hydrolysis of proteins by a microwave equipment is suitable for rapid determination of collagen in meat products.

INTRODUCTION: For the determination of collagen in meat products hydroxyproline is analysed photometrically after acidic hydrolysis of the sample (BGA, 1988; ISO 1977;SOMMER, 1970). The time for hydrolysis takes approximately 8 to 16 hours depending on the hydrolysis liquid (MÖHLER and VOLLEY, 1969) with exception of the hydrolysis method described by ARNETH (1979). ARNETH used perchloric acid as hydrolysis liquid and could reduce the time for hydrolysis to 90 minutes. But the samples have to be homogenized under liquid nitrogen after removing water and fat. So, these methods cannot be used in meat processing enterprises for control of raw material or intermediate products before or during the production process. The analysis time can be reduced by using a commercially available microwave digestor. In this case the time for hydrolysis takes only 30 minutes.

The objective of this investigation was to compare the hydrolysis by microwave irradiation with the method routinely used by the Institute for Meat Hygiene and to test this hydrolysis method for its suitability to determine collagen.

MATERIALS and METHODS:

Materials: Raw beef, ham, sausages, spread, gelatine and mixtures of gelatine with meat products.

Hydrolysis and Hydroxyproline Determination: The microwave hydrolysis was carried out using hydrochloric acid (6 mol/l) and a pressure limit of 85 psi according to the instructions of the producer. The microwave oven was programmed for 20 min at 100%, 5 min at 50% and 5 min at 0% power. The hydrolysis were carried out with and without a pressure control unit. In the first case only one hydrolysis vessel was connected with the pressure control unit. For comparative analysis sulphuric acid (3 mol/l) was used for hydrolysis. The ratio between the amount of the sample and the digestion liquid was identical in both cases. The photometric determination of hydroxyproline was carried out according to ISO-Method 3496.2.

Reagents for color reaction: **Chloramine-T reagent** - Dissolve 1,41 g of chloramine-T in 10 ml of water and successively add 10 ml of propan-l-ol and 80 ml of the buffer solution, pH 6.0 (50.0 g of citric acid monohydrate, 12 ml of glacial acetic acid, 120.0 g sodium acetate trihydrate and 34.0 g of sodium hydroxide/1 000 ml water. Mix this solution with 200 ml of water and 300 ml of propan-l-ol). Prepare this solution immediately before use. **Color reagent** - Dissolve 10,0g of 4-dimethylaminobenzaldehyde in 35 ml of perchloric acid solution [60 % (w/w)l and then slowly add 65 ml of propan-2-ol. Prepare this solution on the day of use.

	STANDARD HYDROLYSIS	MICROWAVE HYDROLYSIS
Sample weight (W)	5 g	2 g
Digestion liquid	40 ml H_2SO_4 (3 mol/l)	15 ml HCl (6 mol/l)
Hydrolysis	16 h at 110°C	30 min
1. Dilution (V_1)	200 ml	100 ml
	Filtration	
	Transfer v_D ml to a flask for 2. dilution	
2. Dilution (V_2)	100 ml	100 ml

STANDARD HYDROLYSIS	MICROWAVE HYDROLYSIS
Color reaction	
Mix	
2 ml (v_P) from 2. dilution or water (for blank)	
+ 2 ml water	
+ 2 ml chloramineT solution (leave 20 min at room temperature)	
+ 2 ml color reagent (heat 20 min at 60°C)	
Absorbance measurement at 558 nm after cooling to room temperature	

Micro procedure. Use 100 µl (v_P) of the 1. dilution and 3.9 ml water for color reaction instead of 2 ml from the 2. dilution and 2 ml water.

Calculation: Prepare at least 4 hydroxyproline standard solutions so that the concentration of hydroxyproline in the color solution is between 0.3 and 2.4 µg/ml. Carry out the color reaction and measure the absorbance at 558 nm against the blank solution. Plot the absorbance of the standard solution against the concentration of hydroxyproline in the color solution and calculate the concentration in the sample using formula (1) or (2).

Standard procedure

$$\%\text{Hydroxyproline} = \frac{V_1 \cdot V_F \cdot V_2 \cdot c_{Hyp} \cdot 100}{v_D \cdot v_P \cdot W \cdot 10^6} \quad (1)$$

Microprocedure

$$\%\text{Hydroxyproline} = \frac{V_1 \cdot V_F \cdot c_{Hyp} \cdot 100}{v_P \cdot W \cdot 10^6} \quad (2)$$

c_{HYP} - concentration of hydroxyproline in the colour solution; V_F - Volume of colour solution

RESULTS and DISCUSSION: The results of this comparative study are given in Table 1. In contrast to the standard procedure the advantage of the hydrolysis of proteins using a microwave oven is the very short hydrolysis time of only 30 minutes. Thus the whole analytical procedure for the determination of collagen takes approximatly two hours. The comparison of the mean values of the hydroxyproline contents obtained by the described techniques shows only slight differences. The relative standard deviations are between 2 and 11%. These values are comparable with the results of BENGER et al.(1969) and GÜNTHER (1969). There exist no significant differences using the WILCOXON U-test ($P > 0.05$; SACHS, 1984). The recoveries of the hydroxyproline mean values were 102.1% for the standard procedure and 101.6 for the microwave hydrolysis. Also this difference of the recoveries is not statistically significant (U-test; $P > 0.05$).

For pressure control only one hydrolysis vessel was connected to the pressure control unit as a reference. There was no possibility to check, whether the pressure had reached the necessary level in the other vessels, which could account for sporadically obtained very low recoveries.

This hydrolysis method was also tested without using the equipment for pressure control. The power settings were not changed for this procedure. In this case all 12 vessels have to be placed in the microwave oven filled with the sample or the same volume of hydrolysis liquid, if the number of samples is less than 12, in order to prevent an excessive rise of the pressure.

The analysis speed could be further increased using a small volume (e.g. 100µl) of the first dilution of the hydrolysate for colour reaction instead of carrying out a second dilution.

At least the recommendation of the International Oragnisation for Standarization should be observed regarding to the differences between the results of two determinations. This difference shall not exceed 5 % of their arithmetic mean, if the determination is carried out simultaneously or in rapid succession, by the same analyst.

CONCLUSIONS: This comparative investigation demonstrates that the hydrolysis of proteins by a microwave equipment is suitable for rapid determination of collagen in meat products. The microwave procedure takes only 3% of the hydrolysis time usually required, total collagen analysis could be determined within two hours. The hydroxyproline values obtained by using both hydrolysis methods were statistically identical.

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REFERENCES:

ARNETH, W. (1979): Untersuchungen zur schnelleren Eiweißhydrolyse für die Hydroxyprolinbestimmung in Fleisch und Fleischwaren. Fleischwirtsch. 75, 24-27

BENGER, H., POYER, I., LORENZ, H. and SCHINZEL (1969): Genauigkeit der Bestimmung von 4-Hydroxyprolin in Fleisch- und Wurstwaren. Z. Lebensm. Unters. u. Forsch. 139, 201-204

BGA (1988): Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG. Bd.I/2, L 06.00-8, Beuth Verlag Berlin Köln

GÜNTHER, H. (1969): Zur Bestimmung von Hydroxyprolin in Fleischwaren. Fleischwirtsch. 65, 505-510

ISO-International Organisation for Standardization (1977): Meat and Meat Products - Determination of Hydroxyproline, Method Nr.3492.2

MÖHLER, K. and VOLLEY, W. (1968): Zur Analytik von Hydroxyprolin und Glutaminsäure in Fleischerzeugnissen. I. Mitteilung: Einfluß der Hydrolyse. Z. Lebensm. Unters. u. Forsch. 140, 189-199

SACHS, L. (1984): "Angewandte Statistik - Anwendung statistischer Methoden". 6. Auflage, Springer Verlag Berlin Heidelberg New York

SOMMER, H. (1970) Zur Bestimmung des Hydroxprolingehaltes in Fleischwaren nach Stegemann. Fleischwirtsch. 66, 837-839

Table 1: Comparison of standard and microwave hydrolysis

	x (% Hyp)	V (% Hyp)	s (% Hyp)	rel s (%)	med (% Hyp)	min (% Hyp)	max (% Hyp)	diff (%)
Beef (n=12)								
Standard	0.243	±0.0177	0.0128	5.3	0.240	0.226	0.271	5.3
Microwave	0.256	±0.0364	0.0285	11.1	0.253	0.227	0.309	
Ham (n=12)								
Standard	0.104	±0.0146	0.0114	10.0	0.104	0.083	0.123	1.0
Microwave	0.109	±0.0084	0.0060	5.6	0.108	0.102	0.112	
Sausage (n=12)								
Standard	0.504	±0.0320	0.0250	4.9	0.508	0.465	0.541	1.0
Microwave	0.510	±0.0348	0.0272	5.3	0.507	0.465	0.569	
Spread (n=12)								
Standard	0.074	±0.0031	0.0024	3.2	0.073	0.070	0.078	2.7
Microwave	0.076	±0.0032	0.0025	3.3	0.076	0.072	0.081	
Gelatine (n=12)								
Standard	12.932	±0.3156	0.2464	1.9	13.024	12.490	13.255	-3.1
Microwave	12.537	±0.8157	0.6369	5.1	12.601	11.217	13.450	
Gelatine* (n=48)								
Standard	12.765	±0.2837	0.5601	4.4	12.801	11.318	14.365	-0.5
Microwave	12.698	±0.4938	0.9747	7.7	12.835	8.357	14.131	

* Gelatine and meat products with added gelatine calculated as hydroxyproline in the added gelatine.
Hyp - hydroxyproline; n - number of samples; x - mean value; V - confidence interval ($p \leq 0.001$); s - standard deviation; rel s - relative standard deviation; med - median value; min - minimum; max - maximum; diff - difference between standard and microwave hydrolysis, standard hydrolysis = 100%