	<b>Amino acids determination in ham for the identification of added protein hydrolysate</b>	<i>Durchführung</i> <i>Dr. Detlef Timmermann</i> <i>Otto Reichwald</i> <i>Raoul Kafka</i>	Versuchs-Nr. <b>TRK-0814_1</b>
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## 1. Introduction

5 samples of ham were analyzed to determine the amino acid concentrations from NPN and for identification of added protein.

## 2. Materials and Methods

### 2.1. Sample preparation

Exactly 5 g of the sample were weighted into plastic cups, 30 ml ultrapure water and 5 ml ultrapure water with internal standard (Norleucine) were added. Everything was homogenized with an Ultra Turrex in intervals of 30 sec. Afterwards 10 ml 10 % sulfosalicylic acid were added and deposit for 20 min. in a refrigerator, finally filtered through a micro filter.

### 2.2. Hydrolysis with CEM Discover microwave system

500 µl of the filtrate was pipetted in a glass vial (1,5 ml volume), put into the vessel body with 7,5 ml 6N HCL.


The hydrolysis vessel was put inside the microwave system, hydrolyze at 150° C for 17,5 min.

At the end of the reaction the hydrolysis vessel was let cool down, the sample was dried in a block heater.

1 ml sample dilution buffer was added to the dry samples to get dissolved for analyzing and filtered using a membrane filter ( MembraSpin, 0,22 µm ).

## 3. Amino acid analysis

For determination of amino acids the Amino Acid Analyzer Aracus from MembraPure was used. The methods for amino acid analysis are based on ionchromatographic separation of the amino acids.

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#### 4. Results

Table 1 shows the amino acid concentration of hydrolyzed NPN from ham. In sample 1 the amino acid concentrations are increases in comparison of the results from the Sample 2, 3, 4 and 5. The producer of the ham (sample 1) added gelatin in the ham during the production.

**Table 1:**

Amino acid concentration of hydrolyzed NPN in mg / 100 g ham						
Amino acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Asp	74,6	13,2	15,8	19,3	14,2	
Thr	52,4	15,1	11,6	13,3	11,5	
Ser	46,3	17,2	16,3	14,5	16,6	
Glu	132,1	79,1	60,9	58,6	53,1	
Gly	141,3	43,3	25,1	32,8	44,5	
Ala	94,8	42,7	31,8	41,4	31,3	
Val	56,2	14,0	11,2	5,3	18,6	
Met	21,3	3,2	6,1	8,4	7,5	
Ile	37,9	7,8	8,0	16,7	9,7	
Leu	61,5	17,2	15,6	28,4	15,4	
Tyr	32,7	8,9	9,7	8,1	9,9	
Phe	28,6	9,7	9,2	8,9	9,5	
His	306,1	312,2	296,4	301,0	330,7	
Lys	107,4	26,8	21,0	23,9	22,3	
Arg	76,8	17,9	12,6	16,0	11,7	
Pro	83,7	25,9	16,4	27,9	23,6	
Hypro	34,3	13,5	12,9	11,7	9,7	
Hlys	5,9	n.n.	n.n.	n.n.	n.n.	

#### 5. Conclusion

An addition of animal protein hydrolysate (gelatin) increases the amino acid concentrations of hydrolyzed NPN. Especially telling are the levels of hydroxyproline and hydroxylysine. They are markers for added gelatin in the ham. When using gelatin in ham a declaration in the list of ingredients is mandatory in any case.