Determination of chitin content in insects

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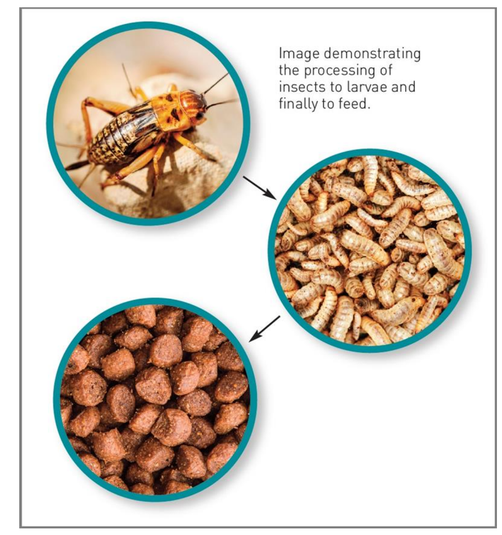
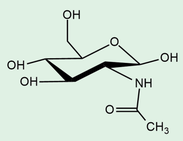
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

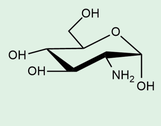
Introduction and research aim



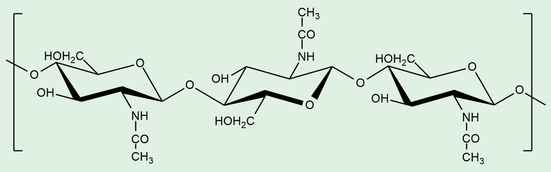
Various insect-derived foodstuffs are often heralded as sources of protein for the diet. Edible insects may contain a high amount of protein, but true protein levels can be overestimated when the substance chitin is present. In insect protein research, it seems that when extracting protein from insects, chitin may be also extracted, which may interfere with most protein analysis. Quantification of chitin is therefore becoming increasingly important in food and feed analysis. Chitin is a high molecular polymer and it is insoluble in water and most solvents, so direct quantification of chitin is almost impossible. The conventional methods for the quantification of chitin content are based on its hydrolysis, and the assessment of the monomer, glucosamine. The rate-limiting step of this chitin analysis is the preparation of chitin hydrolysates. The maximum hydrolysis of chitin and glucosamine recovery can be obtained under conditions of 6-8 M hydrochloric acid at 100-110 °C for 4-13 hours. The purpose of this work was to develop a rapid microwave hydrolysis method for preparation of chitin hydrolysates.

Materials and methods





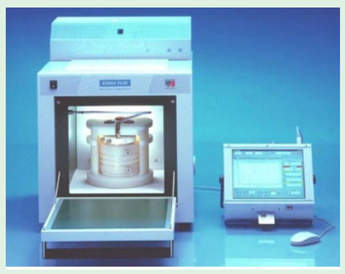
Materials: N-acetyl-glucosamine (NAG) and crab chitin (98%) were used as standards

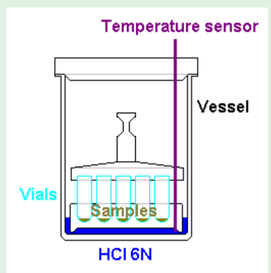


Chitin hydolysis

The first step of sample preparation is acidic hydrolysis of chitin.

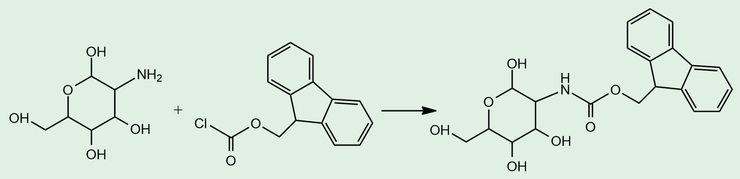
Twenty five milligrams of the samples were hydrolysed by **6 N HCL (4 hours)** in a Milestone ETHOS One Microwave digestion system. Hydrolysates were completed to 3 ml by distilled water.





Derivatization

The N-acetyl-glucosamine (NAG) obtained after the hydrolysis is reacted with FMOC reagent at pH 11.4 and the derivatized form is measured by HPLC. After derivatization with FMOC, glucosamine elutes in 2 peaks (α and β isomers)



Glucosamine FMOC FMOC-glucosamine

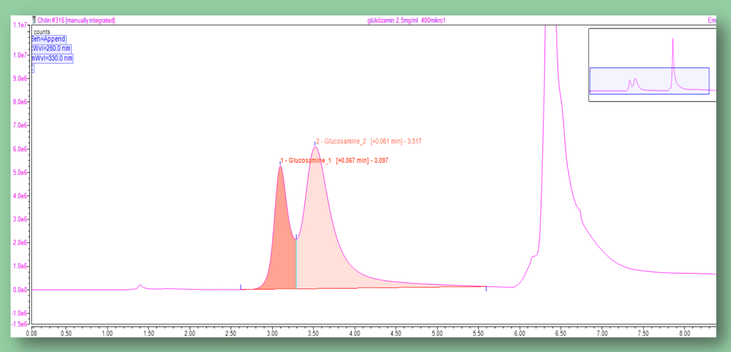
HPLC method

The Thermo Scientific Vanquish (USA) HPLC equipment and a NUCLEOSIL 100-5 C18 NAUTILUS (150 / 4.6) column were used to determine glucosamine concentration. FD parameters: λex = 260 nm λem = 330 nm. The column temperature was set to 30 ° C. Separation was performed by gradient elution, the two eluents were A: 0.05% TFA (pH 2.30), B: ACN, flow rate 1.2 ml / min.

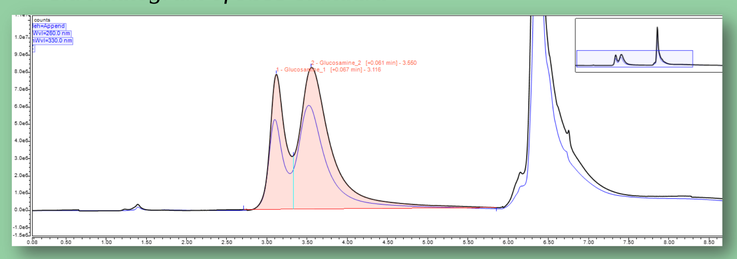
Results and discussion

Specificity

NAG elutes in 2 peaks, the sum of the two components giving the total NAG values.



Chromatogram of NAG standard

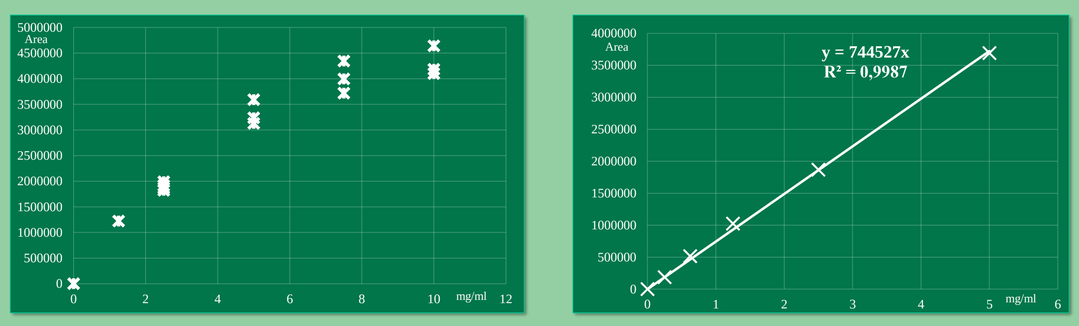


Chromatogram of NAG standard and hydrolysed crab shell chitin

Validation of method

Linearity and range

The measurement is linear over the concentration range of 0.005 to 0.85 mg / ml.



Calibration curves of N-acetyl-glucosamine

Accuracy

Recovery test of hydrolysation: 72%

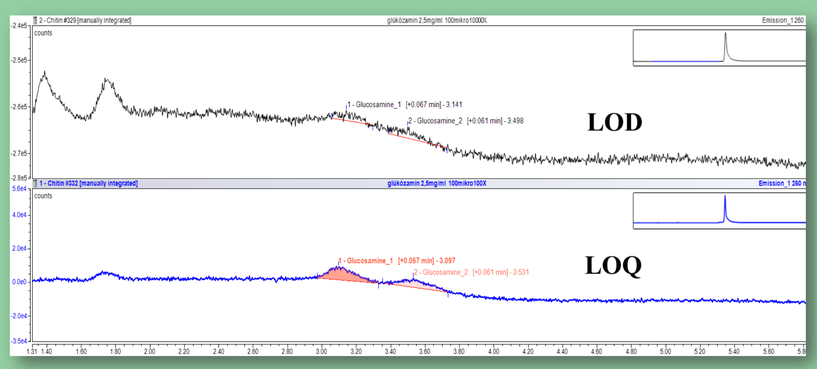
Recovery test of derivatization

Sample	Spiked amount (mg)	Recovery (%)
Crab shell chitin	4,2	75,2
	40	72,8

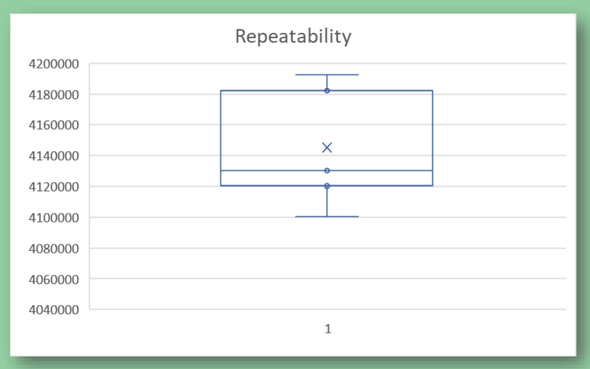
Percent recovery of 10 and 100 % of added NAG

Detection (LOD) and Quantitation Limit (LOQ)

LOD was determined as a Signal to noise ratio 3:1; LOD = 0,0006mg/ml
LOQ was determined as a Signal to noise ratio 10:1; LOD = 0,0020 mg/ml

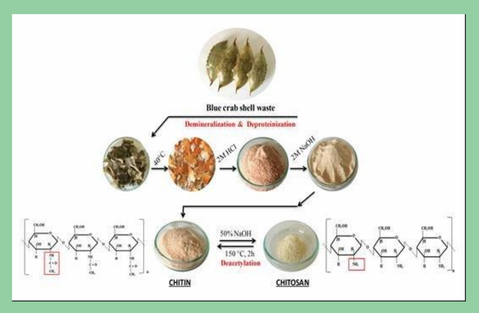


Precision
Repeatability



Concentration (mg/ml)	Area	Average	SD %
2,5	1821379		
2,5	1866807		
2,5	1916286		
2,5	1988412	1898221	3,2
10	4100273		
10	4120273		
10	4130273		
10	4182402	4116940	4,5

The purpose of this work was to develop a rapid microwave hydrolysis method for preparation of chitin hydrolysates. During acid hydrolysis of chitin, the concentration of HCl, temperature, and heating time are prime attributes affecting glucosamine recovery. The optimum conditions of microwave hydrolysis of chitin were found at 100 °C with 6 M hydrochloric acid for 4 hours. The recovery of chitin over 70 %, as described in the literature.
To determine insect chitin a derivatization-based RP-HPLC method was adapted and validated.



References:

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- Zhu, X., Cai, J., Yang, J. & Su, Q. (2005): Determination of glucosamine in impure chitin samples by high-performance liquid chromatography. Carbohydr Res 340(10):1732-8.
- Zhou, J.Z., Waszkuc, T. & Mohammed, F. (2005): Determination of glucosamine in raw materials and dietary supplements containing glucosamine sulfate and/or glucosamine hydrochloride by high-performance liquid chromatography with FMOC-Su derivatization: collaborative study. J AOAC Int 88(4):1048-58.