

QUANTIFICATION OF NEURAMINID ACIDS IN MEAT BY HPLC-BASED FLUORESCENCE DETECTION USING AN ENZYMATICALLY SYNTHESIZED INTERNAL STANDARD

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Abstract – Herein we describe a HPLC-based method for analysing the neuraminic acid content in meat. A synthetic sialic acid derivative (Indoxyl-Gal- α -2,6-N-propionylneuraminic acid) was used as an internal standard. The synthesis of this compound via a chemoenzymatic pathway and its hydrolytic stability was characterized. Samples of red meat were spiked with this component and treated under sialic acid-releasing conditions. The released neuraminic acids were then derivatized, analyzed by HPLC, and the Neu5Ac/Neu5Gc content of the meat sample was calculated by comparison with the internal standard. Various meat samples were analyzed by this method for the quantification of N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). This methodology will find application in investigating the effects of Neu5Gc in red meat.

Key Words – Neu5Ac, Neu5Gc, N-glycolylneuraminic acid, red meat

I. INTRODUCTION

Sialic acids are a family of carbohydrates commonly found as terminal residues of cell receptors while rarely appearing in their free form [1,2]. To date, the two best known being N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid 2 (Neu5Gc, Figure 1):

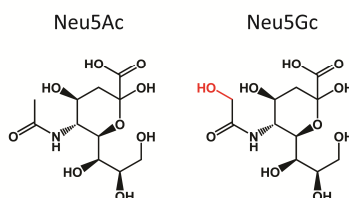


Figure 1: Most common forms of sialic acids

In contrast to most mammals, humans are not able to generate Neu5Gc due to the lack of the required biosynthetic pathway. However, Neu5Gc from dietary sources - originating mainly from red meat products - has been found to be incorporated into human tissues [3]. Furthermore, humans have been found to express anti-Neu5Gc antibodies [4], and for this reason, chronic inflammation resulting from an immune response to dietary Neu5Gc. To better understand the role of dietary sialic acids, a method for their quantification in meat product is required.

II. MATERIALS AND METHODS

The synthesis of NHS-activated propionic acid is based on the procedure described by Lapidot et al. [5]. The chemoenzymatic synthesis of Indoxyl- α -2,6-N-propionylneuraminic acid was performed as described previously [6]. Residual indoxyl-Gal was removed after incubating the samples for 30 min at 37 °C by adding β -galactosidase from *E. coli* (10 U). Buffer salts, enzymes, co-substrates and reaction by-products from the crude reaction mixtures were removed using reversed-phase solid phase extraction.

Meat samples were minced with an electric kitchen blender and 100 ± 2 mg samples of the homogenate were weighed into 1.5 ml centrifuge tubes, and aqueous acetic acid (1 ml, 2M) and the Indoxyl-Gal-Neu5Pr internal standard solution (20 μ l, 0.4 mM) were added. The mixture was incubated at 80 °C for 4 h before being centrifuged (18000 g, 10 min, 4 °C). The top 500 μ l of the clear supernatant were transferred to a fresh tube and the acid was removed by centrifugal evaporation. The dried sample was then dissolved in water (200 μ l), centrifuged again (18000 g, 10 min, 4 °C), and derivatized with *o*-phenylenediamine: red meat samples was performed with *o*-phenylenediamine (10 mg/ml in a 30 μ l solution of 200 mM NaHSO₃) at 80 °C in the absence of light. After 4 hours of reaction time, the solution was analyzed by HPLC using a C18 reversed-phase column; phase A was pure water, phase B was acetonitrile, phase C was methanol. The flow speed was 1 ml/min, and fluorescence was measured at the excitation/emission wavelengths of 373/448 nm.

III. RESULTS AND DISCUSSION

The conditions determined above were applied to determine the Neu5Ac and Neu5Gc content of a number of red meats. Samples of each meat were combined with known quantities of Indoxyl-Gal-Neu5Pr and were maintained at 80 °C for four hours in 2 M solutions of acetic acid. The products of this treatment were analyzed and the Neu5Ac and Neu5Gc contents of each meat were quantified by comparison to the internal standard: The analyzed red meats showed the following sialic acid contents (Neu5Ac μ g/g tissue, Neu5Gc μ g/g tissue \pm s.d.): pork (68 ± 3 , 15.2 ± 0.7), beef (69 ± 8 , 36 ± 5), lamb (46 ± 2 , 33 ± 1), rabbit (59 ± 2 , 0.4 ± 0.4), and hare (50 ± 4 , 1 ± 1).

IV. CONCLUSION

The results show that, of all the red meats studied, beef has the highest Neu5Gc content in absolute terms, while lamb shows the highest Neu5Gc content as a proportion of total Neu5Ac and Neu5Gc content. In agreement with previous reports, negligible levels of Neu5Gc are present in rabbit meat [7,8] or in the meat of the closely related hare.

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