



SIALIC ACID (NEU5AC AND NEU5GC) LEVEL IN INDIAN MEAT AND POULTRY PRODUCTS BY LC-MS/MS

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ABSTRACT

There are more than fifty naturally occurring derivative of sialic acid have been described. Out of this only two are predominant sialic acid, one is n-acetylneuraminic acid (Neu5Ac) and the other is n-glycolyneuraminic acid (Neu5Gc). Recent studies are reported that Neu5Gc is a carcinogenic compound which is present highly in meat and milk products. In this study, we aim to find the level of sialic acid in Indian meat and poultry products by using LC-MS/MS, Especially the estimation of carcinogenic Neu5Gc in meat and poultry products. The study shown that chicken and egg shown sialic acid only in the form of Neu5Ac and absence of Neu5Gc. Meat products shown presence of sialic acid in both forms.

KEY WORDS

Sialic acid, Neu5Ac, Neu5Gc, Meat, LC-MS/MS

INTRODUCTION:

Meat is considered as highly nutritious and has become integral part of human diet being a high source of valuable proteins, vitamins (B12, B3 and B6), minerals, micronutrient and fats. Meat is the rich source of few important molecules like creatine, carnosine etc. In India the poultry meat consumption is increasing every year. In 2010 the meat consumption was 2645.9 kilotons and increased to 3246.8 kilotons in 2014, the consumption was increased 22 % in the past five years [1]. However, the meat and meat products contribute high quality of proteins and other nutrient to human diet, recent research studies strongly evidenced that it has carcinogenic molecule i.e n-glycolyneuraminic acid (Neu5Gc) one of the sialic acids.

Sialic acid is a group of compounds consisting of N- and O-substituted derivatives of neuraminic acid, a nine carbon polyhydroxaminoketoacid sugar (5-amino-3, 5-dideoxy-D-glycero-D-galacto-nonulosic acid). More than 50 naturally occurring derivative of sialic acid have been

described [2] and out of this two are predominant sialic acid, one is n-acetylneuraminic acid (Neu5Ac) and the other is n-glycolyneuraminic acid (Neu5Gc). Humans are unable to synthesise Neu5Gc due to chromosomal frame shift deletion in CMP-Neu5Ac hydroxylase (CMAH) gene [3]. But the incorporation of Neu5Gc into human tissues is possible through diet especially red meat and milk products. This incorporation of Neu5Gc into human glycoconjugates could potentially lead to health risk because Neu5Gc can be antigenic when linked to glycoconjugates. [4]. Non- human sialic acid (Neu5Gc) that differs by one oxygen atom from the human sialic acid (Neu5Ac) and there are many reports evidenced that the incorporation of Neu5Gc may generate chronic inflammation and contribute to the diet related carcinomas [5,6,7,8,9] and other diseases in human like haemolytic-uremic syndrome (HUS) [10]. The non-human sialic acid found in many numbers of human cancers including breast, throat, lung uterine, throat, ovarian, cervical and liver.

The objective of the present study was to use LC-MS/MS to quantify Neu5Ac and Neu5Gc in red meat and poultry products. Our results have provided that important information into the risk level of dietary intake of Neu5Gc from red meat like pork, beef and lamb. The study also revealed the level of Neu5Ac content in poultry products like chicken meat, egg.

EXPERIMENTAL PROCEDURE:

Chemicals and reagents:

Reference standard Neu5Ac (99.9%) and Neu5Gc (99.9%) were purchased from Sigma Aldrich, USA. Ultrapure LC-MS grade methanol was purchased from J. T. Baker. LC-MS grade ammonium formate procured from Biosolve chemicals Netherlands and formic acid from Fluka, Germany. All the analysis was carried out with Milli Q water.

Sample collection:

The sample of pork, beef, lamb and chicken were purchased from local meat market in Whitefield, Bangalore, India. The collected samples were stored in deep freezer at -20°C until the analysis carried out. The poultry eggs were purchased from local market before the analysis.

Sample Preparation:

Before analysis taken the sample were removed from the freezer and bring down to room temperature. From each meat samples one to five grams were taken chopped and homogenised. Prior to estimate sialic acid meat samples were hydrolysed with different acid with different temperatures and time.

Sialic acid determination:

Sialic acid in dairy and food products were determined by our earlier reported LC-MS/MS method [11]. Major amount of sialic acid is bound to glycoprotein, oligosaccharides and glycolipids in meat and poultry products. Samples were hydrolysed with dilute hydrochloric acid (HCl) to make free form of sialic acid from their conjugates.

Meat products like lamb, Beef, pork and Chicken were hydrolyzed using 50 mM HCl acid at 80°C for 60 min. Agilent 6460 ESI (-Ve) LC-MS/MS with mass hunter workstation software version B.02.1 was used for data acquisition. Thermo hypersilgold C-18 (150 mm X 4.6 mm ID X 3 μ) column was used. A gradient pump programme was used between 0.3 percentage of formic acid in water and 5 mM of ammonium formate in 90

percentage methanol and 10 percentage water. The optimized MRM transition for Neu5Ac was 308.1 to 87 m/z , fragmentor at 62 V, collision energy was set as 8V, for Neu5Gc the transition was 324.0 to 116.0 m/z , fragmentor and collision energy was 74 and 12 V respectively.

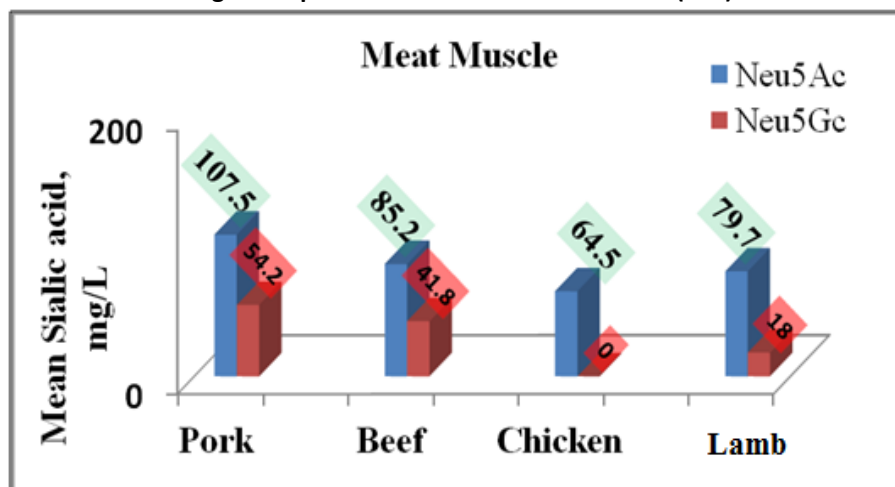
RESULTS AND DISCUSSIONS:

Meat and egg products:

Sialic acid concentrations were analyzed in Indian meat and egg products. Egg yolk and egg white were separated and tested for sialic acid content. Egg yolk shown high level, 802 ± 66 mg/L of sialic acid in the form of N-acetylneuraminic acid (Neu5Ac) whereas egg white contains only 239 ± 12 mg/L of Neu5Ac. Egg white & yolk does not show the presence of N-glycolylneuraminic acid (Neu5Gc).

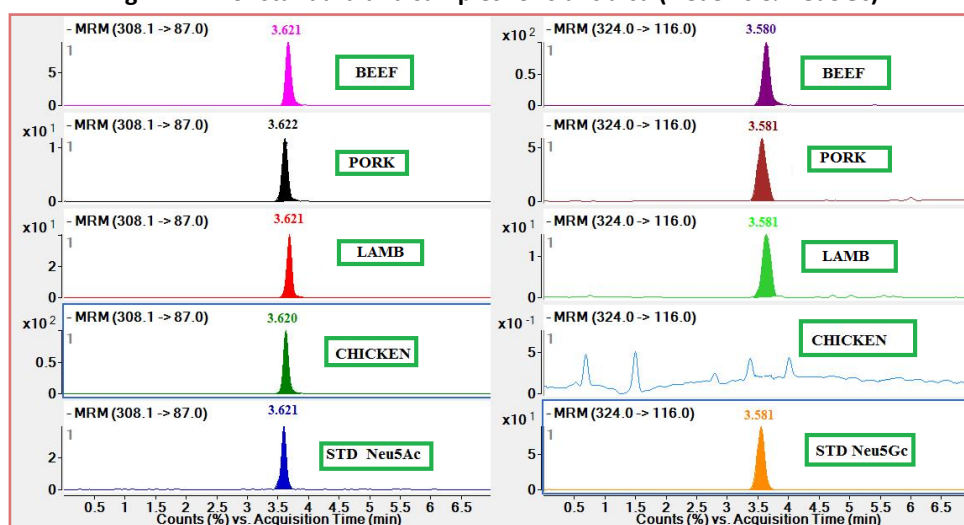
Among various meat products like beef, lamb, chicken and pork samples were analysed for the sialic acid content, chicken shows sialic acid only in the form Neu5Ac, but the other three products had the sialic acid in the form of both Neu5Ac and Neu5Gc. Different parts of meat like liver, muscle and fat in lamb, chicken, pork, and beef were analysed for sialic acid content. Result indicates that liver has maximum level of sialic acid in the all the tested meat products. The results of mean sialic acid in meat and egg products were shown in Table. 1.

Among different meat products analysed for sialic acid content pork muscle shown 161.7 ± 8.7 mg/Kg sialic acid followed by beef muscle 127.2 ± 6.1 mg/Kg, lamb muscle 97.7 ± 10.7 mg/Kg and chicken muscle 64.5 ± 8.5 mg/Kg. In chicken muscle, sialic acid present predominantly 100 percentages in the form of Neu5Ac and Neu5Gc was absent. Lamb muscle sialic acid content was 81.6 percentage in the form of Neu5Ac and remaining 18.4 percentage was in the form of Neu5Gc. Beef muscles and pork muscle shown 32.9 percentage and 33.5 percentage of Neu5Gc respectively. Comparison of sialic acid present in meat muscle sample is shown in Fig 1. The Fig 2 represents the chromatogram of Neu5Ac (308.1 > 87.0) & Neu5Gc (324.0 > 116.0) in reference standards, chicken & meat products MRM (multi reaction monitoring). For chicken there were no peak response obtained for the transition of 324.0 > 116.0 (Neu5Gc) in the chromatogram while the other meat products shown presence.

Fig 1 Comparison sialic acid in meat muscle (n=6)

Table. 1: Sialic acid concentration in Indian meat and egg products (n=6)

S.No	Product	Mean Neu5Ac, mg/Kg	Mean Neu5Gc, mg/Kg	Mean total sialic acid, mg/Kg
01	Chicken liver	602.3	ND ^a	602.3
02	Chicken muscle	64.5	ND ^a	64.5
03	Egg Yolk	802	ND ^a	802
04	Egg white	237	ND ^a	237
05	Lamb muscle	79.7	18	97.7
06	Lamb liver	298.3	72.5	370.8
07	Lamb fat	34.2	14.9	49.1
08	Pork liver	143.5	71	214.5
09	Pork muscle	107.5	54.2	161.4
10	Beef muscle	85.2	41.8	127
11	Beef liver	120.5	52.2	172.7

^a Not detected

Fig 2 MRM of standard and samples for sialic acid (Neu5Ac & Neu5Gc)


CONCLUSION:

This study has established the level of sialic acid in Indian meat & poultry products like beef, chicken, lamb,

pork and egg. Further the study revealed the level of Neu5Ac and Neu5Gc in each product. In chicken and eggs has sialic acid in the form of Neu5Ac and it shown absence of Neu5Gc, a suspected carcinogenic molecule.

Egg yolk shown highest level of sialic acid (Neu5Ac) compare to other products. Pork muscle has highest percent level of Neu5Gc followed by beef in the total sialic acid level. Level of sialic acid (Neu5Ac & Neu5Gc) in organ (liver) high compare to muscle which is comparable to Suna et al [12]. Further detailed studies are required to find the level of sialic acid in cooked meats along with spices.

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