Detection and specific identification of giblets in processed chicken meat products using droplet digital PCR based quantification of miRNAs

Vishnuraj M.R.[1], Suresh Devatkal[1], Vaithiyanathan S.[1], Mendiratta S.K.[2], Ch. Srinivas[1], Barbuddhe, S.B.[1]

[1] ICAR-National Research Centre on Meat, Chengicherla, Hyderabad, Telangana, India,500092.
[2] ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttarpradesh, India, 243122.

Introduction: Incorporation of less costly offal meat in to processed meat products for revenue maximization and without proper ingredient declaration amounts to food fraud, which has strict restrictions under EU commission. Hence, there is a strict requirement for regulatory bodies to identify the proportions of offal tissues incorporation, for which no robust molecular methods are currently available. In instances of offal identification, available DNA based analytical methods are inappropriate because all the tissues have exactly the same DNA sequence and which can't be used for declaration of "true to its labelling". Hence, this study aimed to identify tissue specific miRNA markers to detect the presence of giblets (liver, heart and gizzard) in chicken meat products.

Materials and methods: We performed deep sequencing of chicken microRNAs from liver, heart, gizzard, muscle & blood using Illumina Nextseq 500. For samples of liver, heart, gizzard, muscle & blood, 11, 25, 9.2, 8.8 & 6.1 million reads were analyzed respectively. Unique and differentially expressed miRNAs were evaluated and miRNAs having differential expression in each tissue were selected as potential candidate markers. Candidate miRNA markers tested using Locked Nucleic Acid (LNA) miRNA primer (Exiqon, Qiagen, USA) in QX200 Automated digital droplet PCR (Bio-Rad, USA) using EVAGreen chemistry. Annealing temperature and primer concentrations were optimized using 20 µL ddPCR reactions in a gradient set-up. Specificity of miRNA primers were confirmed through melt curve analysis using SYBR Green based qPCR. Further, these candidate biomarkers were validated in tissues as such and in minced chicken meat samples (raw and cooked) admixed with 5, 25 & 50 % of liver, heart and gizzard separately.

Results: Eight potential candidate miRNA markers were selected based on unique and differential expression in individual tissue types. The ddPCR assay for testing the miRNA markers was standardized for annealing temperature, primer concentration and further optimized for low sensitivity. The LoDabs and LoDrel were reported to be 2.5 copies/µL and 0.94 copies/µL. On investigating the meat mixtures, separate target copy number cut-off values were established for each miRNA targets in raw as well as cooked samples. The miRNA-ddPCR assay detected gizzard using gga (Gallus gallus)-mir-148a -3p, gga-mir-126 -5p and gga-mir-490 -5p, heart using gga-mir-218 -5p and gga-mir-499 -3p and liver using gga-mir-122 -5p in raw as well as cooked samples at 25 g/100 g substitution level.

Conclusion: The miRNA-ddPCR methodology was developed and validated for accurate, robust and reproducible detection of giblets incorporation in processed chicken meat products. The selected miRNA markers and the developed assay established in this study can be a valuable tool for food regulators for accurate detection of tissue origin of meat products in samples including highly processed products.